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## ORIGINAL ARTICLES

### STUDIES IN FOCAL INFECTION*

PRELIMINARY REPORT


BY R. N. DE NIORD, M.D., F.A.C.P., AND B. J. BIXBY, M.D., BUFFALO, N. Y.


DIAGNOSIS

MANY excellent papers have been written and much scientific research work has been carried on by scores of investigators in the effort to determine and to clarify the relationship of focal infections to systemic disease. Due to the preponderance of evidence, the facts which have been adduced should be fully accepted by both the medical and dental professions. But unfortunately there are many in both professions, who have failed to accept the so-called "focal infection theory" either in whole or in part. The reasons for this lie in prejudice, lack of ability to draw conclusions from the overwhelming facts and data already presented, or unfortunate failures in practice due to incomplete search for and eradication of primary foci. We propose to present in this paper a broad and yet concise view of focal infection as a source of systemic disease, and particularly to present an invariable diagnostic factor by which the presence of focal infection may be determined.

The subject of focal infection is not new. As early as 1789, Eyerlin of Christiana in his Materia Rheumatica ad Tonsillitis, considered the relationship of diseased tonsils to rheumatism as clinically evident. In 1818, Benjamin Rush[1] laid before the American Society of Ophthalmology and Oto-Laryngology at a meeting in Cleveland, Ohio, a case of rheumatism of the hip accompanied by toothache, the rheumatism being promptly cured by extraction of a decayed tooth. From Rush's paper in 1818 up to 1909, a review of medical literature reveals the fact that no scientific proof of the connection between focal infection and systemic disease had been published.


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[1]Medical Inquiries, Benjamin Rush, 1818.

Nevertheless it was clinically evident that certain infectious diseases followed tonsillitis. And it was furthermore shown that the same organisms were present in the tonsils and in the secondary lesion; up to this time the clinical evidence was so positive and even overwhelming, that scientific proof seemed scarcely necessary. In spite of this evidence, however, the majority of the medical profession remained sceptical up to a few years ago. During the past ten years, however, many scientists working independently and in different fields, have presented such an abundance of evidence that it would seem that the truth of the thesis had been established, particularly as the data were based on both clinical evidence and experimental and research work. In spite of this there is a large percentage of both medical and dental professions who are still disposed to doubt. And it is with the hope of removing this scepticism and to establish a standardized routine examination by which the presence of focal infection may be detected, that the following studies of changes in the chemistry of the blood are presented. We wish to express our appreciation of the efforts of the American workers, which have made this study possible, such men as Folin, Benedict, Meyers, Van Slyke, and others. We present here nothing new as to methods of examination, but we do present a new interpretation of the findings brought out through the results of their work. It has been found in a series covering several hundred cases, that certain changes invariably take place in the blood chemistry of patients having a focal infection. The findings in these cases may be modified to a certain extent by previous medication and dietary restrictions prescribed for the most part by careful observers working from an empirical standpoint. But when these forms of treatment are known to have been given, the diagnosis may still be made with a high degree of accuracy.

Under the heading of Focal Infection may be included chronic accumulations of pus, and areas exhibiting an abnormal degeneration of the cellular elements, enclosed in any of the cavities or tissues of the body. Most prominent among these tissues are the tonsils, including all lymphoid tissues in Waldeyer's ring, teeth and periodontal tissues, nasal accessory sinuses, middle ear, mastoid and lateral sinuses: in fact all cavities communicating with the mouth, nose, and nasopharynx. The consensus of opinion seems to give the tonsils the place of primary importance as foci of infection, with the teeth and periodontal tissues as second in importance. The list of the most common diseases in which focal infection may prove to be of paramount etiological significance are as follows:

1. Neurasthenia
2. Neurosis
3. Insanity (Cotton)
4. Neuritis
5. Neuralgia
6. Myalgia
7. Myositis
8. Arthritis-acute
9. Arthritis-subacute
10. Arthritis-chronic
11. Gout
12. Osteitis
13. Periostitis
14. Basedow's disease
15. Chorea
16. Meningitis
17. Septicemia
18. Furunculosis

19. Herpes zoster
20. Urticaria
21. Erythema nodosum
22. Myocarditis
23. Endocarditis
24. Pericarditis
25. Arteriosclerosis
26. Bronchitis
27. Pneumonia
28. Asthma
29. Diabetes-mellitus
30. Diabetes-insipidus
31. Nephritis
32. Pyelitis
33. Cystitis
34. Gastric neurosis
35. Gastric ulcer
36. Duodenal ulcer
37. Appendicitis
38. Colitis
39. Cholecystitis
40. Cholangitis
41. Dacrocystitis
42. Conjunctivitis
43. Iritis
44. Corneal ulcer
45. Retinal hemorrhage
46. Optic neuritis
47. Diplopia
48. Glaucoma (?)
49. Possible adjuvant in production of cancer.

Regarding the last named possible effect of focal infection it has been noted that an infected mouth frequently shows a tendency to an acid reaction, this acid reaction favoring cell degeneration of a malignant type following chronic irritation (Mayo).

Rosenow, who has done so much to establish the relationship between focal infection and systemic disease, has said, "In the light of our present knowledge, the argument that infections in the mouth are so common in the mouths of individuals in apparent health, does not minimize their importance. These or other foci are so common in patients suffering from arthritis, neuritis, appendicitis, ulcer of the stomach, cholecystitis, goiter, etc., and so rare in individuals who have had superb health for years that their direct etiologic rôle can scarcely be questioned. The most common location of the focus or source of infection is in the mouth, and the teeth as well as the tonsils must always be carefully examined with this object in mind. However the question of the focus of infection is a matter not only for the stomatologist or the dentist, but also for the general practitioner, the internist and the surgeon. In fact, every branch of medicine needs to be taken into consideration in order to trace the focus from which the organisms gain entrance to the body. The focus of infection must be regarded not only as a place of entrance for the bacteria, but also the place where the organisms acquire the peculiar properties necessary to infect."

The diagnosis of these areas frequently offers one of the most difficult problems in medicine. We find, for instance, many evidences of infection in the mouth in areas whose degeneration is not sufficiently pronounced to be macroscopic,—which have not reached the stage of definite abscess formation with a well-defined walled-off area of pus. Such areas, however, invariably present microscopic evidence of inflammatory changes. The bone may exhibit rarefaction, or condensation, or may only show a change in the arrangement of the cancellous spaces. Their variation in density or structure from the normal bone surrounding them may be so slight as to readily escape

detection. To our minds these areas are even more dangerous than the areas marked off by a layer of dense bone or a pyogenic membrane, as they allow toxins to be absorbed freely into the circulation. These areas of diffuse rarefying osteitis and diffuse condensing osteitis, or the areas exhibiting an alteration in the cancellation of the bone, produce virtually the same leucocytic infiltration, the same degeneration of tissue and blood elements, the same liberation of split proteins, as does the well-defined abscess. The toxic effects of split proteins need not be taken up here: it has been thoroughly investigated by Victor C. Vaughan, ("Poisonous Proteins"). Too often has disappointment for both practitioner and patient followed a diagnosis which was incomplete rather than incorrect. For not only in the mouth, but also in other parts of the body, is a definite diagnosis very difficult to make. And it is safe to say that the major part of the present scepticism regarding the focal infection theory rests on this point.

The blood is the one tissue of the body which is in intimate contact with all the other tissues. Into it are eliminated all the products of the breaking down of tissue, both the normal katabolic end products and those substances which are produced as the result of disease. To the blood, even more than to the urine, we must look in our study of the body, whether in health or disease. The tissues of the body including the blood, consist of cells and intercellular substance. When any tissue breaks down, either completely or in degenerative change, there is an abnormal liberation of the chemical units of which that tissue is made up, each tissue and its parts having their characteristic chemical units. However, all cells no matter what their composition, have nuclei which have the same chemical foundation, in that they consist largely of nucleo-proteins. These are conjugated proteins, being compounds of one or more proteins with nucleic acid. Nucleic acid upon decomposition yields phosphorus and purine bases. In the body the usual end-product of the decomposition of nucleo-protein is uric acid, which is usually classified as *endogenous*, originating in the breaking down of the nuclear portion of tissue elements, and *exogenous* derived from the breaking down of ingested food containing the purine nucleus. A further subdivision is now found necessary for the endogenous uric acid, namely, *normal*, that originating in normal tissue metabolism, and *abnormal*, that originating in nuclear degeneration. Other nitrogenous end-products of metabolism or of tissue degeneration are urea, ammonia and creatinine. And it is interesting to note that each of the four nitrogenous end-products has a different threshold of elimination by the kidney. At present, however, we will consider only that substance, which is of nuclear origin; namely, uric acid, remembering that its presence in the blood may be traced to the normal metabolic processes, ingestion of food rich in purin bodies, or the abnormal breaking down of cellular elements. The main fact to which we wish to call attention is that infections, whether producing pus or not, give rise to a nuclear degeneration of a proteolytic type from the nuclei of the cells so broken down, the end-product of which is uric acid.

Uric acid is eliminated with some difficulty by the kidneys and consequently tends to accumulate in the blood when produced in larger quantities

TABLE I

The cases so reported in this series are those in which the Urea Nitrogen—Total non-proteid Nitrogen and Creatinine fell within the normal limits, the Urine findings running parallel with the blood in that there was no gravity fixation and at the most showed a faint trace of Albumin. We will report therefore at this time only the Uric Acid findings.

| NAME | AGE | SEX | URIC ACID IN MGMS. PER 100 C.C. | DIAGNOSIS | TREATMENT | RESULTS AND URIC ACID FINDINGS | |
|---|---|---|---|---|---|---|---|
| E. T. S. | 34 | M | 4.3 | Erythema nodosum | A. Tooth extraction<br>B. Tonsillectomy | Improved<br>Recovered | 3.2<br>1.6 |
| B. J. | 56 | F | 3.2 | Sciatica | Tooth extraction | Recovered | 1.4 |
| J. Mc. | 34 | F | 3.5 | Arthritis (sub-acute)<br>Neurosis | A. Tooth extraction<br>B. Appendectomy | Improved<br>Recovered | 3.1<br>1.5 |
| C. M. J. | 47 | M | 3.7 | Psychoneurosis | Tooth extraction | Recovered | 1.5 |
| F. H. | 40 | F | 4.5 | Beginning Glaucoma | Tooth extraction | Recovered | 1.6 |
| A. R. | 31 | F | 3.9 | Corneal ulcer | A. Tooth extraction<br>B. Tonsillectomy | Improved<br>Recovered | 3.1<br>1.7 |
| R. J. | 59 | F | 3.7 | Intercostal neuralgia | A. Tonsillectomy<br>B. Tooth extraction | Improved<br>Recovered | 3.1<br>1.5 |
| N. A. T. | 44 | F | 4.2 | Gout | Tooth extraction | Recovered | 1.6 |
| M. B. | 14 | F | 3.4 | Chorea | Tonsillectomy | Recovered | 1.6 |
| C. J. | 45 | M | 4.1 | Debility and neurosis | A. Tooth extraction<br>B. Appendectomy | Improved<br>Recovered | 2.8<br>1.4 |
| J. K. | 31 | M | 3.8 | Neuritis and psycho-neurosis | A. Tonsillectomy<br>B. Tooth extraction | Improved<br>Recovered | 2.4<br>1.6 |
| E. K. | 43 | F | 4.3 | General neuritis and neurosis | A. Tooth extraction<br>B. Tonsillectomy<br>C. Cholecystectomy | Improved<br>Improved<br>Recovered | 3.4<br>2.4<br>1.4 |
| I. T. | 41 | F | 3.7 | Arthritis (sub-acute) | A. Tooth extraction<br>B. Tonsillectomy<br>C. Fistula in Ano | Improved<br>Improved<br>Recovered | 3.4<br>2.6<br>1.5 |
| F. A. H. | 33 | M | 3.6 | Neurasthenia | Tooth extraction | Recovered | 1.3 |
| C. P. | 35 | M | 3.8 | Myalgia | Tooth extraction | Recovered | 1.2 |
| A. Mc. | 37 | F | 4.2 | Arthritis | A. Tooth extraction<br>B. Tonsillectomy | Improved<br>Recovered | 3.7<br>1.6 |
| L. L. | 23 | F | 3.4 | Arthritis and neurosis | A. Tooth extraction<br>B. Tonsillectomy | Improved<br>Recovered | 3.1<br>1.4 |
| D. L. J. | 38 | F | 4.2 | Cervical neuralgia | Tooth extraction | Recovered | 1.6 |
| J. S. | 57 | M | 4.1 | Psychoneurosis and Myocarditis | Tooth extraction | Recovered | 1.3 |
| W. H. D. | 61 | M | 3.7 | Optic Neuritis and Myocarditis | Tooth extraction | Recovered | 1.2 |
| H. H. | 46 | F | 3.3 | Neuritis and secondary anemia | A. Tooth extraction<br>B. Tonsillectomy | Improved<br>Recovered | 2.5<br>1.4 |

than normal over a period of time. Examination of the urine may thus for a long period of time show no more than the normal content of uric acid, while at the same time the blood chemistry reveals a uricacidemia.

It must, of course, be kept in mind that other agencies than chronic infections may produce uricacidemia, as for instance leukemia, primary anemia, cachexias, massive doses of x-ray and radium, etc. All of these conditions are susceptible of diagnosis and elimination. And when so eliminated the presence of a high uric acid content in the blood may safely be taken as indicating that somewhere in the body there is in progress an unusual breaking down of nuclear material. All chronic infections are divided into primary and secondary. And while the secondary infection is usually the source of anxiety to the patient, the primary or inaugurating infective focus is the point of interest to the diagnostician. The presence of a high blood uric acid does not of course point to any particular part of the body as its source. Hence the diagnosis of focal infection made through this medium does not indicate that the teeth or tonsils or any other one organ is at the bottom of the disturbance. But it does point to the necessity for a thorough search for all possible primary foci, and it gives definite authority for the removal of such foci when found.

Practice based upon this assumption has been most happy in the results which it yields. Patients invariably show such a measure of improvement as is to be expected according to their history, and the regenerative power of the tissues which are the site of the secondary infections. Moreover when improvement does not occur, or is incomplete, the possible continuance of focal infection will be revealed by a continued high uric acid in the blood. For the uric acid will not return to its normal level in the presence of primary foci of infection, and on the other hand when these foci are completely eliminated, the uric acid content of the blood will be found to come with the normal range.

## CONCLUSIONS

1. Clinicians have at their disposal well established methods of the estimation of uric acid.

2. High uric acid value in the blood is indicative of nuclear degeneration, which in turn may mean focal infection.

3. Other factors productive of high uric acid, aside from nuclear degeneration, are comparatively easy to determine, i.e., leukemia, primary anemias, cachexias from whatever cause, and massive doses of x-ray or radium.

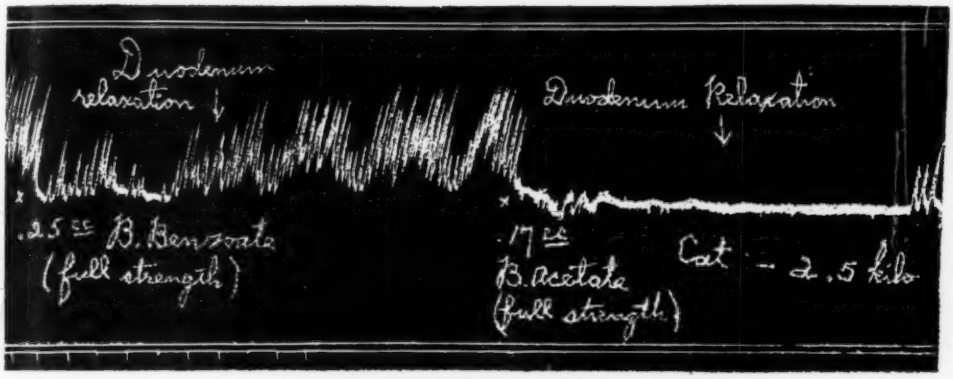
4. Elimination of all foci of infection invariably is followed by a return of the uric acid to normal.

5. Failure to eliminate all foci will prevent the return to a normal uric acid, and this substance therefore furnishes a reliable index to the complete elimination of foci of infection.

# FURTHER OBSERVATIONS OF THE PHARMACOLOGY OF BENZYL COMPOUNDS.* II.

By Carl Nielsen and John A. Higgins, Chicago, Ill.

IN the course of our pharmacologic investigations of benzyl compounds we have endeavored to establish the relative efficiency of various benzyl esters and other benzyl compounds as intestinal relaxants. Our work has included benzyl esters of melting points both below and above room temperature.



Figs. 1 and 2.—Show a comparative action of benzyl benzoate and benzyl acetate on the cat's duodenum.

These were all supplied by the Chemical Research Department of The Abbott Laboratories, and have been described chemically by Volwiler and Vliet.[1] In a previous article[2] we presented records obtained with liquid benzyl esters mainly, pointing out that our results suggest a correlation of rate of benzyl

*From the Pharmacologic Department of The Abbott Laboratories, Chicago.
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hydrolysis and smooth muscle relaxant property in the cases of simple benzyl esters. In further support of this theory, we present records (Figs. 1 and 2) of comparative action on the cat's duodenum of benzyl benzoate and benzyl acetate. In Fig. 1 the doses injected were equal in benzyl content; in Fig. 2

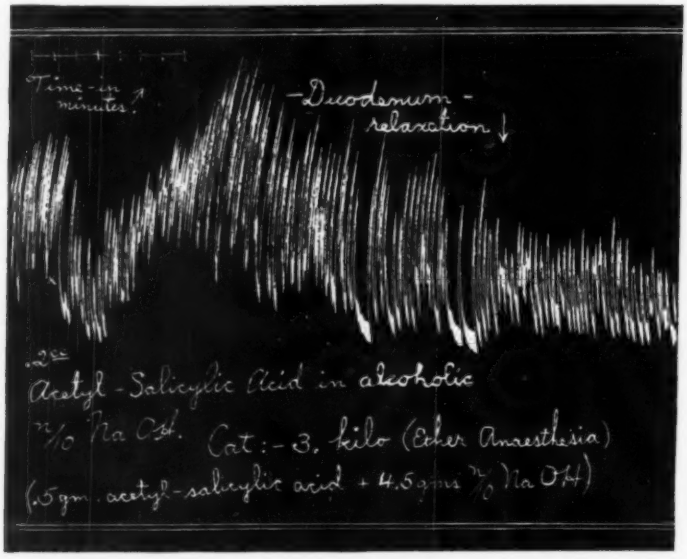
Time - in minutes
Duodenum - relaxation
.2cc
Acetyl - Salicylic Acid in alcoholic
½% Na Oh. Cat:- 3. kilo (Ether Anaesthesia)
(.5 gm. acetyl-salicylic acid + 4.5 gms ½% Na OH)

Fig. 3.—Shows the action of acetylsalicylic acid on the duodenum of a cat.

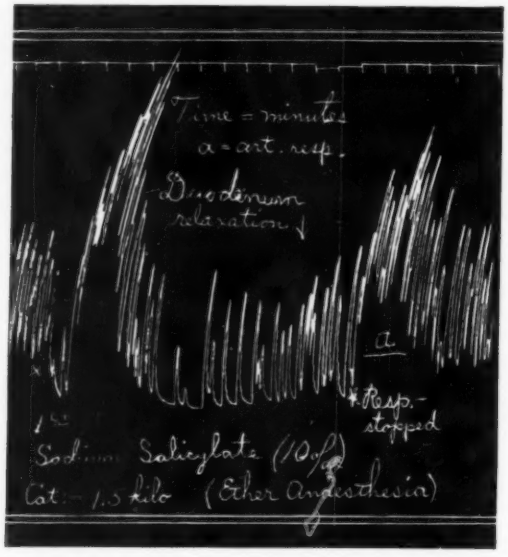


Fig. 4.—Shows the action of sodium salicylate on the duodenum of a cat.

the dose of benzyl acetate was one-half by volume of that of benzyl benzoate.

Benzyl salicylate and benzyl acetylsalicylate, which both contain substituting groups, appear as exceptions to this rule. These two esters were found to produce a relaxation of the intestine, *in situ*, greater than expected from their benzyl content and rate of benzyl hydrolysis.

We have, therefore, carried out a series of experiments to determine whether the relatively powerful relaxant action on smooth muscle of these two benzyl esters may be ascribed in part to their acid radical. Our method of procedure was, to test the action of other salicylates, as well as acetylsalicylic acid, upon the intestine *in situ*, following the same technic as that used in the case of the esters.

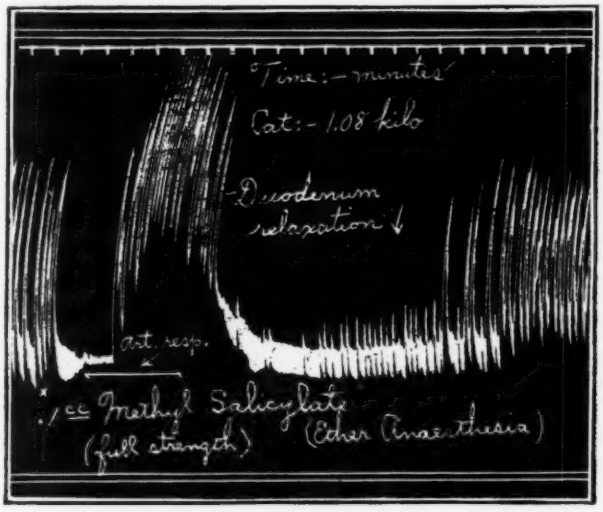


Fig. 5.—Shows the action of methyl salicylate on the duodenum of a cat.

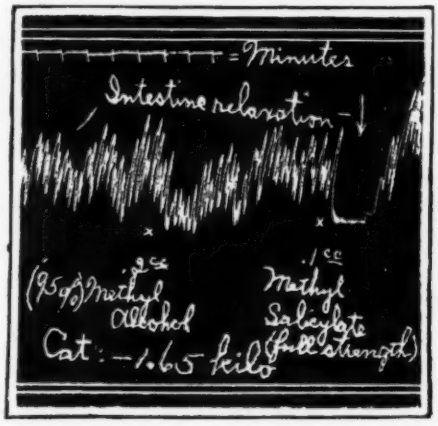


Fig. 6.—Shows a comparative action of methyl alcohol and methyl salicylate on a cat's intestine.

Fig. 3 shows the result obtained on the duodenum of a cat from the injection of 0.2 c.c. of a 10 per cent solution of acetylsalicylic acid in tenth-normal alcoholic sodium hydroxide. The contraction which followed the immediate primary relaxation was probably due to the depression of respiration. As the respiration returned to normal, it will be noted that a secondary and more lasting relaxation appeared. The amount of alcohol injected doubtless interfered somewhat with the reaction, but it was nevertheless chosen as a solvent because of the low solubility of acetylsalicylic acid in oil.

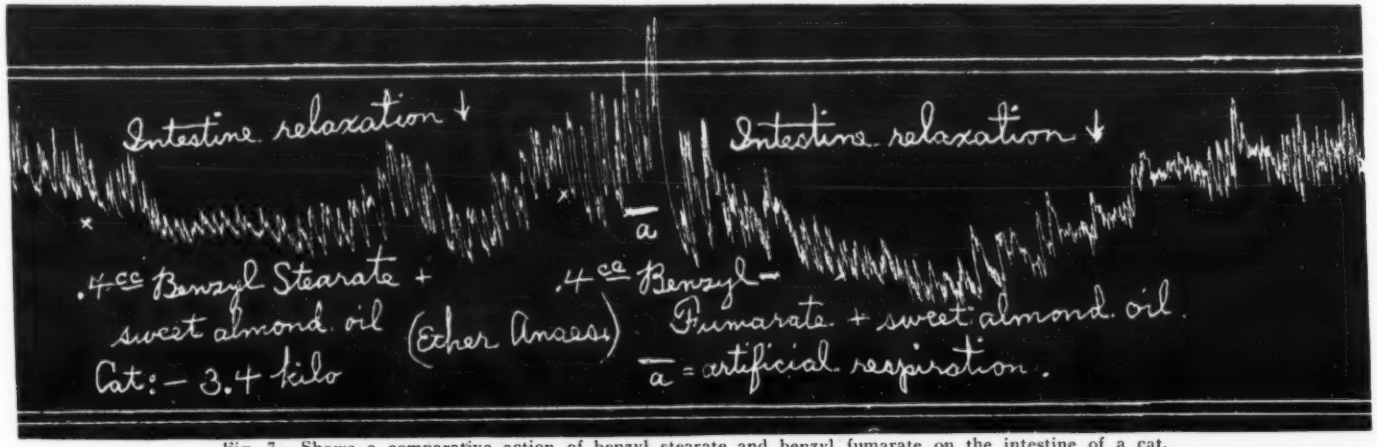


Fig. 7.—Shows a comparative action of benzyl stearate and benzyl fumarate on the intestine of a cat.

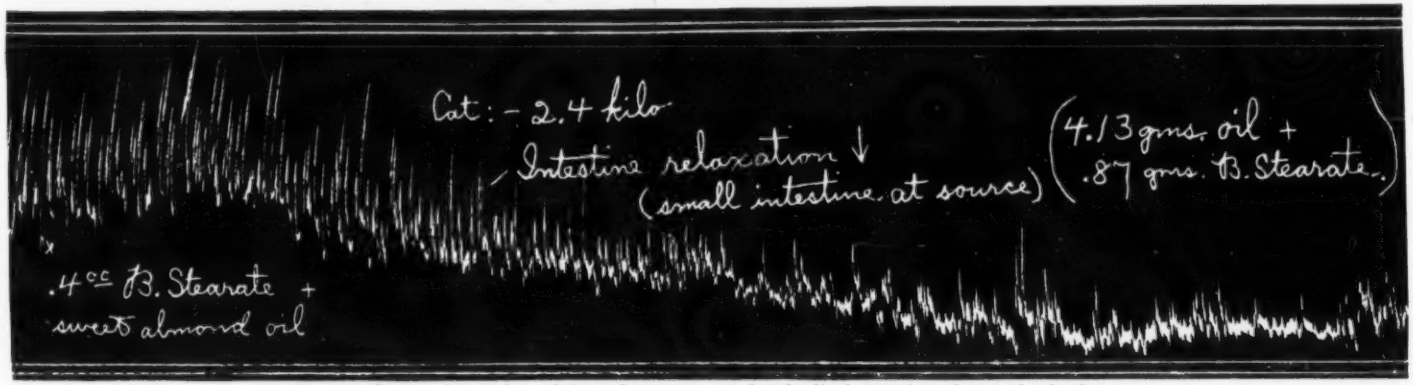


Fig. 8.—Shows the action of benzyl stearate on longitudinal muscles of a cat's duodenum.

Fig. 4 demonstrates the action on the duodenum of a cat of 1 c.c. of a 10 per cent sodium salicylate. Here again we note a primary relaxation followed by a contraction due to respiratory depression, and with the gradual return of normal respiration, a secondary relaxation of the duodenum.

Fig. 5 shows the effect on the duodenum of a cat from an injection of 0.1 c.c. of methyl salicylate. Since the relaxation produced by this compound was so pronounced, a test was made to compare the action of methyl alcohol with that of methyl salicylate.

Fig. 6 shows that, while methyl alcohol did relax the intestine, the methyl salicylate, containing less methyl in the molecule, produced a decidedly greater relaxation.

Benzyl salicylate, when treated with alcoholic potassium hydroxide, in

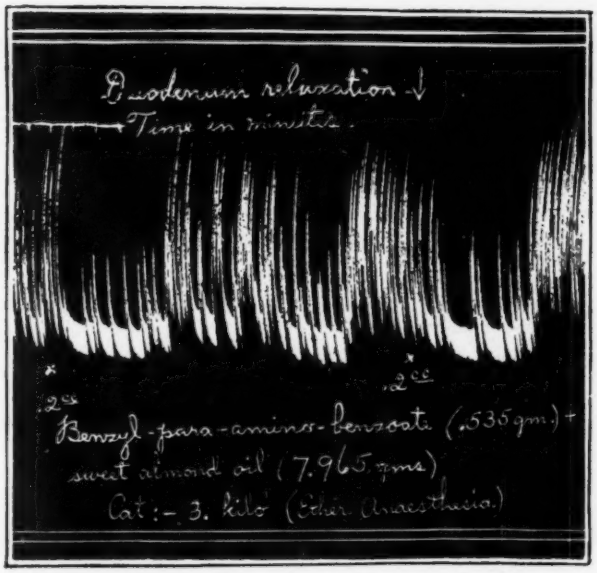


Fig. 9.—Shows the action of benzyl para-amino benzoate on the duodenum of a cat.

vitro, has the lowest rate of hydrolysis of all the benzyl esters we have investigated. The rate of hydrolysis of the benzyl group in benzyl acetylsalicylate does not exceed that of the salicylate (See Volwiler and Vliet, Journal of American Chem. Soc., 1921, *43*, 1672). Since their power to relax smooth muscle of the intestine is greater than that of simple benzyl esters possessing a much higher rate of hydrolysis, it appears reasonably certain that this greater efficacy is due to their difference in structure from the simple benzyl esters. This difference consists in the molecule of the salicylate containing a hydroxyl group and that of the acetylsalicylate, a substituted hydroxyl group. Our records demonstrate, on the other hand, that these acids alone do not possess any striking power to relax the intestine. It seems evident, therefore, that the action of these two benzyl esters is not the ensemble of their molecular constituents, but rather a more potent one possessed by the intact molecules.

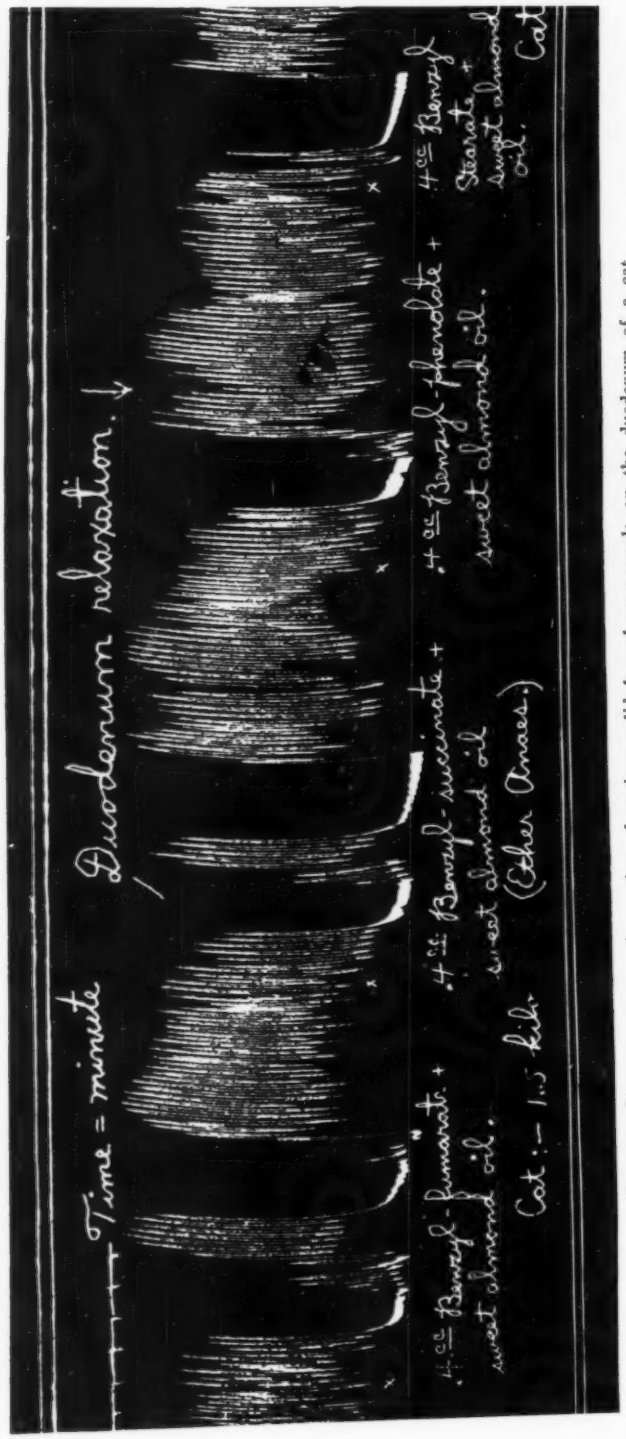
Time = minute
Duodenum relaxation.
4 cc. Benzyl-fumarate + sweet almond oil.
4 cc. Benzyl-succinate + sweet almond oil
4 cc. Benzyl-phenolate + sweet almond oil.
4 cc. Benzyl Stearate + sweet almond oil.
Cat :- 1.5 kilo.
(Ether Anaes.)
Cat

Fig. 10.—Shows a comparative action of various solid benzyl compounds on the duodenum of a cat.

In our investigation of benzyl esters of higher melting points (solid above room temperature), we have used the Trendelenburg method,[3] unless otherwise specified. The benzyl esters were dissolved in sweet almond oil in such amounts that the various solutions used for injection contained the same proportion of the benzyl radical. Thus, for instance, 0.87 gm. of benzyl stearate dissolved in 4.13 gm. of oil correspond in benzyl content to a solution of 0.35 gm. of benzyl fumarate in 4.65 gm. of oil.

Fig. 7 presents a comparison between benzyl stearate and fumarate on the intestine of a cat. It shows that benzyl fumarate produces a greater relaxation than benzyl stearate when injected in less than half the amount by weight.

Fig. 8 is a record taken by the Jackson method,[4] showing the rather

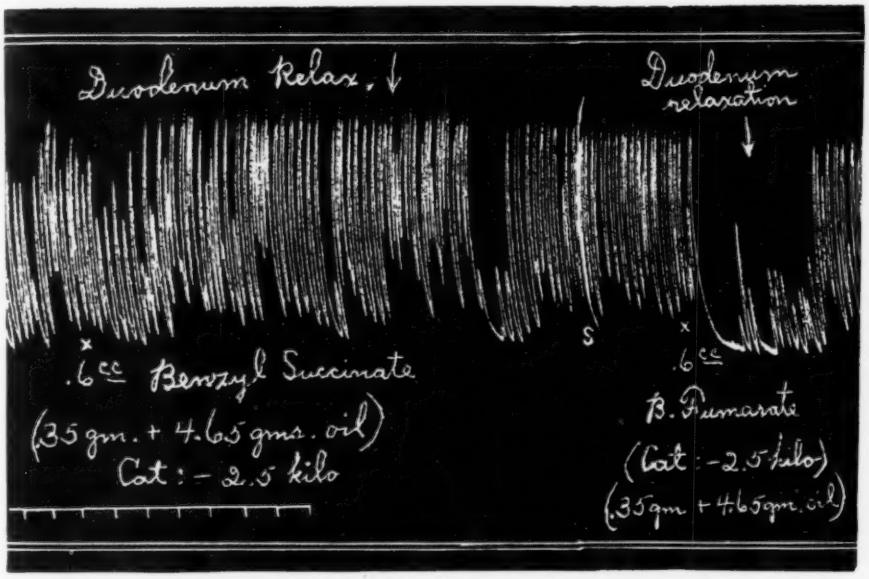


Fig. 11.—Shows a comparative action of benzyl succinate and benzyl fumarate on the cat's duodenum.

slow relaxation of the small intestine of a cat produced by benzyl stearate.

The action of benzyl para-amino-benzoate on the cat's duodenum is shown in Fig. 9. This ester was a fawn-colored crystalline powder, melting between 88.5 and 89.5° C. It was first dissolved in the proportion of 0.535 gm. in 4.465 gm. of oil, to conform in benzyl content with the other benzyl ester solutions, but in this proportion, it did not remain in solution at body temperature. An addition of 3.5 gm. of oil was therefore made.

In our study of comparative efficiency of the solid benzyl compounds, we have had continuous evidence of the regrettable fact that these compounds cannot be administered intravenously in a solvent more suitable than oil. The cat, which we have found to be the animal best suited for our work, naturally presents differences in individual response to this mode of administration. As a rule, the younger animals react more readily than the older. Fig. 10 is a typical example of a readily responding duodenum in a young cat.

The solutions injected were made to conform in benzyl content, with the exception of the benzyl phenolate which contained 1 gm. plus 4 gm. of oil. The relaxation of the duodenum took place only 2 to 3 minutes after each injection, regardless of any differences in the rate of initial hydrolysis of the various

Fig. 12.—Shows the action of a 5 per cent solution of mono-benzyl barbituric acid on the duodenum of a cat.

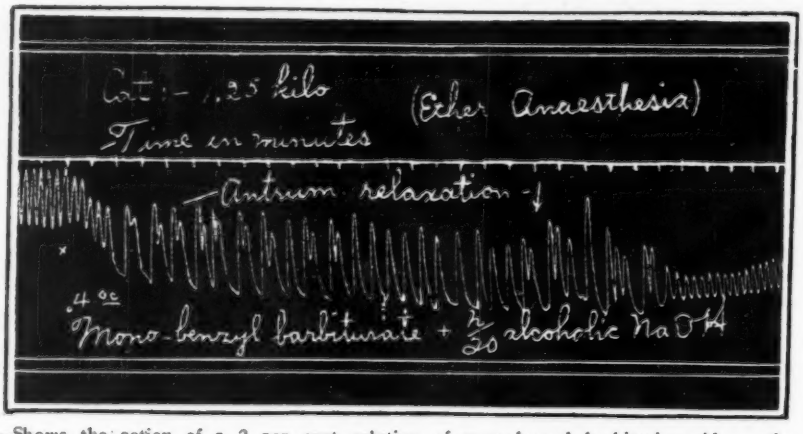


Fig. 13.—Shows the action of a 2 per cent solution of mono-benzyl barbituric acid on the antrum pylori of a cat.

compounds. The recovery was rapid in all instances; the slight difference in the duration of relaxation being the only means of comparison.

As a typical contrast to this tracing we present Fig. 11. This cat was an old female. An injection of 0.042 gm. of benzyl succinate in oil was made. Approximately fifteen minutes elapsed before the first sign of relaxation appeared. The drum was stopped at S. for a period of 45 minutes, after which

time the intestine had recovered from a series of alternate relaxations and contractions. An injection of the corresponding benzyl molecular amount of benzyl fumarate (0.042 gm.) in oil was now made and produced a relaxation in less than one minute. This difference in onset of relaxation corresponds to the difference in the rate of initial hydrolysis *in vitro* of these two esters. Volwiler and Vliet show that benzyl succinate hydrolyzes at the rate of 12 per cent in 5 minutes, 16.8 per cent in 15 minutes and 17.1 per cent in 20 minutes; while benzyl fumarate hydrolyzes at the rate of 35 per cent in 5 minutes, 41.5 per cent in 15 minutes, and 43 per cent in 20 minutes when treated according to their method. After a period of two hours their rates of hydrolysis run closer together, namely, 40 per cent for the succinate and 57 for the fumarate.

We obtained also marked relaxations of the cat's duodenum with other benzyl compounds. The records obtained with benzyl phenolate have already been presented. Benzaldehyde was also included in our experiments.

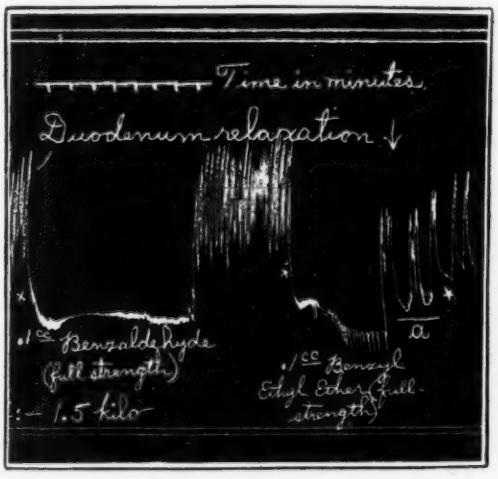


Fig. 14.—Shows the action of benzaldehyde and benzyl ethyl ether on the duodenum of a cat.

Figs. 12 and 13 show the action of monobenzyl-barbituric acid on the duodenum and antrum pylori of a cat. It produced a relaxation of both. On the antrum, the relaxation was preceded by an increase of vigor and decrease of frequency of the movements. The antrum experiment was made according to the Jackson method.[4]

Fig. 14 shows the action of benzaldehyde and benzyl ethyl ether on the duodenum of a cat. The benzyl ethyl ether has a marked depressing effect on the respiration, even upon inhalation. Injected intravenously, it stopped the respiration and produced a gasping effect. This gasping may be noticed in the tracing by the downward strokes of the lever after the duodenum had relaxed. Artificial respiration was applied but failed to revive the animal, the heart having stopped.

Fig. 15 shows the relaxation of the isolated uterus of a virgin guinea pig produced by benzyl ethyl ether and benzaldehyde.

## CONCLUSIONS

Our observations suggest that the relaxing power of benzyl esters on the smooth muscle fibers of the intestine as a rule is dependent upon the benzyl content and upon the rate of hydrolysis of the benzyl compound. In all cases where we have succeeded in obtaining comparative records, with the exception of those of benzyl salicylate and benzyl acetylsalicylate, we have found that the higher the rate of hydrolysis, the greater the efficiency. According to our findings benzyl fumarate is more efficient than benzyl succinate, particularly with regard to initial action; the succinate is more efficient than the stearate, the acetate more powerful than the cinnamate, which in turn is of higher efficiency than the benzoate.

The high relaxing power of benzyl salicylate and benzyl acetylsalicylate, in spite of their slow rate of benzyl hydrolysis, may be attributed to their

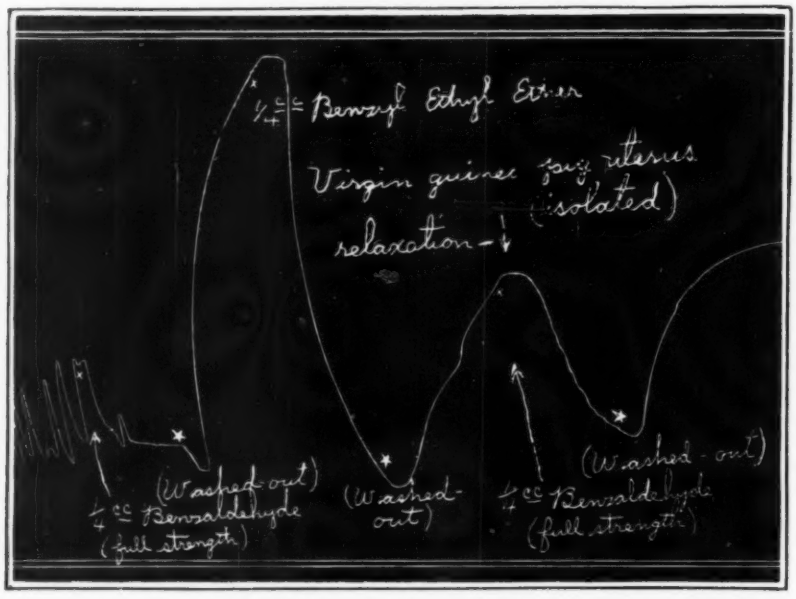


Fig. 15.—Shows the action of benzaldehyde and benzyl ethyl ether on the isolated uterus of a virgin guinea pig.

intact molecules. These compounds differ from other benzyl esters in that their molecules contain hydroxyl or substituted hydroxyl groups. The benzyl acetylsalicylate is more efficient as an intestinal relaxant than the salicylate, in fact it is more powerful than any of the benzyl esters investigated by us.

Various benzyl compounds other than benzyl esters, namely benzyl phenolate, benzyl ethyl ether, and monobenzyl barbituric acid, as well as benzaldehyde, were also found to possess smooth muscle relaxing properties.

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# THE PRESENCE OF BACTERIA AND FORMED ELEMENTS IN THE URINE OF RABBITS*

By Henry F. Helmholz, M.D., and Frances Millikin, Rochester, Minn.

THE occurrence of so-called spontaneous nephritis in rabbits has often been commented on by investigators who use these animals for experimental purposes. Bell and Hartzell have accurately described the pathogenesis of the lesions and have emphasized the necessity for adequate controls when rabbits are used in the study of nephritis. They call attention to the fact that some groups of rabbits may be entirely free from evidences of nephritis, while other groups are affected. In eight instances they attempted to isolate the etiologic organism by culturing pieces of kidney. Cultures from four of the kidneys were negative, two yielded a streptococcus and two a staphylococcus. These organisms, however, when injected intravenously into rabbits, failed to reproduce the disease. No other bacteriologic work was done, the discussion being confined chiefly to the histology and pathology of the affected kidneys.

Helmholz and Beeler, in experiments on urinary infections in rabbits by *Bacillus coli*, found one rabbit with a naturally acquired pyelocystitis. The urine contained pus, and cultures yielded *Bacillus coli communis*. It is worthy of note that this was the only rabbit of a large series whose urine contained bacteria, pus, or other pathogenic elements. The colon bacillus isolated in this case was the one used in subsequent experiments.

Recently, we decided to continue the experiments, which were interrupted by the war, and began to search for another rabbit whose urine contained pus and colon bacilli. Contrary to previous experiences, the urine of a large number of rabbits was found to contain pus, erythrocytes, and albumin or casts associated with bacteria, usually colon bacilli. Furthermore, in accordance with observations of Bell and Hartzell, the condition was more prevalent among certain groups; occasionally, lots were entirely free from urinary indications of pathologic conditions. Necropsies performed in a few instances in which urinary findings had been positive, disclosed lesions of the kidney of the type described by Bell and Hartzell.

Since the more or less common occurrence of colon bacilli in the urine of rabbits introduces an important source of error into experiments on the production of urinary infections by that organism, it has seemed worth while to present some of our observations. These serve to emphasize further the necessity of making careful controls, and of selecting a healthy stock with which to work.

*From the Department of Pediatrics, Mayo Clinic, Rochester, Minnesota.
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TABLE I

BACTERIA AND FORMED ELEMENTS IN URINE WHICH YIELDED A POSITIVE CULTURE

| RABBIT | SEX | LEUKOCYTES IN EACH FIELD (low power) | PUS | OTHER URINARY FINDINGS | FINDINGS FROM CULTURES ON | | | ORGANISMS IN EACH c.c. |
|---|---|---|---|---|---|---|---|---|
| | | | | | DEXTROSE BRAIN BROTH | BLOOD AGAR PLATES | LITMUS LACTOSE AGAR | |
| A-12 | M | Very few | | Many erythrocytes and epithelial cells | *Bacillus coli* and streptococcus | | | |
| A-18 | F | Very few | | | Diplococcus | | Diplococcus | *Few* |
| A-19 | M | Large clusters of 500 | + | | *Bacillus coli* | | *Bacillus coli* | |
| A-24 | F | 200 | + | | *Bacillus coli* and streptococcus | | *Bacillus coli* | 160 |
| A-26 | M | 8 to 10 | | | Streptococcus | | Negative | |
| A-46 | | 100 | + | Many epithelial cells | *Bacillus coli* | | *Bacillus coli* | 100 |
| A-48 | | Large clusters of 200 | + | | *Bacillus coli* | *Bacillus coli* | | 300 |
| A-50 | F | Few clusters of from 8 to 10 | + | | *Bacillus coli* | Negative | *Bacillus coli* | 30 |
| A-51 | F | Very few | | Few erythrocytes | *Bacillus coli* | Negative | Negative | |
| A-55 | | 0 | | Few granular casts | *Bacillus coli* | Negative | *Bacillus coli* | 60 |
| A-64 | | Very few | | Many erythrocytes | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* and diplococcus | 1 per 10 c.c.<br>2 per 10 c.c. |
| A-65 | F | Few clusters of from 2 to 3 | + | | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* | |
| A-69 | F | 8 | + | | *Bacillus coli* | | *Bacillus coli* | |
| A-70 | F | 0 | + | | Green-forming diplococcus | | Contamination | |
| A-73 | F | 5 | | | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* | |
| A-75 | F | Few clusters of 25 | + | | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* | |
| A-79 | F | Few | | | Diplococcus | Negative | | |
| *A-79 (one day later) | F | Clusters of 2 to 3 | | | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* | Many |
| A-80 | F | 0 | | | Staphylococcus | Negative | Negative | |
| A-89 | F | Few | | | *Bacillus coli* Staphylococcus and diplococcus | *Bacillus coli* staphylococcus and diplococcus | *Bacillus coli* and staphylococcus | 150 |
| A-90 | F | Few | | | *Bacillus coli* | *Bacillus coli* | *Bacillus coli* | 130 |

*Necropsy examination revealed a faint red streak in the medulla of the right kidney and *Bacillus coli* in both kidneys.

We examined the urine of ninety-nine rabbits. Pus was found in thirty, casts in three, erythrocytes in three, and colon bacilli not accompanied by any of the foregoing pathogenic findings, in three. The urine of sixty-three of the ninety-nine rabbits was cultured. The specimen of urine was obtained for culturing by catheterization as follows. After the meatus and surrounding area had been washed under a fine stream of 1 per cent lysol pumped from a syphon, a small sterile metal catheter was inserted and the urine collected in sterile tubes. It was cultured in tubes of dextrose brain broth, and on blood agar plates, on litmus lactose agar plates, or on both. The urine of forty-three of the sixty-three rabbits was sterile in all mediums used. The cultures from

TABLE II

FORMED ELEMENTS IN URINE WHICH YIELDED A STERILE CULTURE

| RABBIT | SEX | LEUKOCYTES IN EACH FIELD (low power) | PUS | OTHER URINARY FINDINGS |
|---|---|---|---|---|
| A-21 | F | 3 | + | |
| | | 1 cluster of 11 | | |
| A-29 | F | Few | | Casts |
| A-36 | | 5 to 6 | + | |
| | | 1 cluster of 10 | | |
| A-67 | F | 8 | | One epithelial cast |
| A-72 | M | Clusters of 25 | + | |
| A-74 | M | Many large clusters | + | |
| A-82 | | 50 | + | |
| | | Clusters of 25 | + | |
| *A-83 | | 15 | | |
| | | Clusters of from 4 to 10 | | |
| A-86 | M | 4 to 5 | + | |
| | | Clusters of from 3 to 10 | | |
| A-91 | M | 12 | | Few hyaline casts |
| A-96 | M | 8 | | Few granular casts with albumin |

*Necropsy revealed congestion at the corticomedullary border. Red streaks in papillae. Submucosal tissue of pelvis of right kidney, hemorrhagic.

sixteen revealed colon bacilli, in pure culture in twelve, and mixed with other organisms in four. Four of the cultures revealed streptococci or staphylococci (Table I). Eleven of the forty-three specimens of sterile urine contained pus, albumin, erythrocytes or casts (Table II).

## SUMMARY

A colon bacilluria associated with other pathologic conditions occurs in rabbits more often than is commonly supposed. It introduces a serious source of error into experiments concerned with the production of urinary infections in rabbits, and demands extreme care in selecting a healthy stock for experimental animals and controls.

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# ETIOLOGY OF SCARLET FEVER*

## IV. Variation or Types of the Alkali-Producing Organism in Scarlet Fever

By R. W. Pryer, D.P.H., Detroit, Mich.

THREE previous papers from this laboratory dealing with the bacteriology of scarlet fever have been published in this Journal. In the first two papers no mention was made of spore production, because our early work had led us to the conclusion that no spores were present. The third paper dealt entirely with the cultural and other characteristics of the organism in the spore bearing stage. We are now of the opinion that the young cultures of this spore-producing coccus-like organism are spore free and are also convinced that considerable changes in morphologic and serologic characteristics occur when the organism is carried on artificial culture media under certain conditions. But few instances of spore producing cocci are recorded in our literature and in no instance do these spore producing cocci appear to resemble the one described by us. Our later conclusions herein reported of various mutation or cyclic changes in this peculiar organism have caused us to confine our work very largely to attempts to determine something of these changes and how they may be brought about, since there is considerable direct and indirect evidence that the virus of scarlet fever varies considerably in its clinical manifestations, resistance, etc., from other organisms.

At the present time the belief is prevalent that the discharge from the throat, nose, ear, glands, etc., harbors the virus and that the majority of infections come from this source directly, although indirect infections undoubtedly also occur. In other words, the greatest factor in the dissemination of the disease is the carrier, whether he be a recently discharged case or, what all too frequently happens, a mild unrecognized case of the disease. The presence of the virus in these discharges is so generally recognized and so evident from an epidemiologic study of the disease that little more need be said.

Laboratory proof of the presence of the virus in these discharges, while somewhat contradictory, is fairly conclusive. Stickler[46, 47] by subcutaneous inoculation of children with material from the throat and nose of scarlet fever patients, after treatment of the material with 1 to 600 carbolic acid, apparently caused the disease. Working with monkeys, Bernhardt,[3] Levaditi,[31] Cantacuzene[9] and Greenbaum[21] reported the transmission of the disease following injection of material from scarlet fever patients. These results have been questioned by Draper[15] and Hektoen,[24] Hektoen's work being based

*From the Laboratories of the Detroit Health Department.
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on the feeding of milk contaminated by swabbings from the throat and nose of scarlet fever patients. Using pure cultures of organisms isolated from the throat of a scarlet fever patient, Cantacuzene[10] claims to have produced a condition in monkeys resembling scarlet fever in the human. These latter experiments of Cantacuzene are of exceedingly great interest to us, since it is possible that the organism with which he was working is the same as the one with which we are concerned. G. F. and G. H. Dick[16, 17, 18] working with filtrates from throat and nose mucus and washings, claim that injections of the sterile filtrate into humans fails to transmit the disease. However, they give no idea of the age of their volunteers and this we believe to be an important point, since scarlet fever is primarily a disease of childhood and adults seem to be more or less immune, irrespective of whether or not they have had scarlet fever. Working with a pleomorphic organism G. F. and G. H. Dick have obtained some suggestive results following the swabbing of throats of volunteers with suspension of this organism. It is difficult to judge from the limited amount of information published by them, but it is not at all improbable that the pleomorphic organism with which they are working is similar to the one with which we are concerned. If then these secretions or discharges harbor the virus, what is the nature of it?

One of the old ideas concerning the virus of scarlet fever is that it is extremely resistant. Consequently we believe that we are justified in saying that there is a possibility or probability of the virus being a spore producer. This idea of a resistant virus permeates our medical literature and many physicians who have had a wide experience with scarlet fever have their favorite story of this or that case caused by handling some article (toy, book, clothing, etc.), which had been used by a scarlet fever case a long time previously. It is interesting to note that comparatively few instances of a bacteriologist reporting a spore bearing organism as the cause of the disease are recorded.

One instance of a spore bearing organism present in scarlet fever is the work reported from this laboratory,[45] and we believe this organism to be the cause of the disease, although in this instance Koch's postulates have not as yet been fulfilled.

Since it is the purpose of this paper to show that the spore variety is the final variety, or form in the development of the organism and since this question is one of considerable concern as well as dispute among bacteriologists, it has been thought well to refer to certain papers which deal with mutation or cyclic growth among bacteria.

That scarlet fever is a very variable disease both as regards its clinical manifestations and the intensity of different outbreaks is commonly recognized. It is essentially a disease of childhood and almost 90 per cent of the cases are under the ten year age group. It is most common in winter and is almost unknown in tropical countries. Since the different forms of the disease will concern us directly, reference is made to our standard works in medicine for descriptions of these forms.

The number of so-called pleomorphic organisms which have been de-

scribed in scarlet fever is comparatively limited. Class[11, 12, 13, 14] made several reports concerning a pleomorphic coccus. The inclusion bodies of Döhle[19, 20] as reported by him and partially at least confirmed by Kolmer,[28] Nicoll,[40, 41] Nicoll and Williams,[42] and Bernhardt,[3] may possibly be considered as a variable organism, although Glomset[22] and others claim that they are without significance in the disease. The protozoan-like bodies described by Mallory[32] are apparently variable. The organism with which we are working is so extremely variable that it has seemed best to cover briefly the situation regarding variation in bacteria as brought out by different investigators.

### VARIATION, MUTATION OR CYCLIC GROWTH OF BACTERIA

The question of variation in bacteria is one of fundamental importance and deserves much more attention than has been paid to it. It is not within the province of this paper to enter into a discussion of the terms to be used, although the writer believes that the expression pleomorphism fails to explain the results so ably described by Jones,[27] Mellon,[33, 34, 35, 36] Bergstrand,[1 2] Löhnis[29, 30] and others. On the other hand, in the light of our present knowledge are we justified in using the expression "life cycle of bacteria?" Personally we feel that the expression "life cycle" might better be reserved for such organisms as plasmodium malaria, etc., in which part of the transformation or cycle occurs in an intermediate carrier, usually an insect or animal other than the human.

The use of the term "mutation" applied to bacteria has not been generally accepted and must be considered as indicating some unusual change in the properties of an organism.

The expression, *variety* will be used in this paper in speaking of the different forms of the organism which we believe causes scarlet fever.

The appearance of budding forms in pure cultures of bacteria have been reported by many observers. The more recent papers of Löhnis[30] and Bergstrand[2] refer to most of these reported instances and should be consulted. Wade and Manalang[53] report the presence of budding and branching forms in B. influenza. Heinemann[25] and others have reported observations on the assumption of coccoid forms by Parke strain No. 8 of B. diphtheria, while branching forms of the diphtheria organism have been reported by several workers. Hort,[26] using a method of single cell study devised by himself, claims that bacteria such as B. typhosus multiply by branching, budding and by the production of gonidial bodies in addition to the more common method of fission. This view is also held by Jones, Mellen, Bergstrand and many others. Jones working with Azotobacter has observed all these reproductive forms in pure cultures. As regards Azotobacter cultures, approximately the same views are held by many workers who have published their reports in recent years.

In regard to variation in serologic characteristics, considerable data is being presented all the time. Instances of change in agglutination characteristics in cultures or portions of cultures as well as in carriers of the disease are fairly numerous. We are all familiar with the fact that a freshly isolated

culture of B. typhosus is frequently inagglutinable until it has been transplanted many times. Benians[8] has apparently produced an inagglutinable Shiga dysentery by injection into rabbits of a suspension of agglutinable Shiga dysentery in tragacanth. The work of Bordet[5] on changes produced in B. coli by animal passage must also be mentioned.

To sum up then, there is considerable evidence that many of our common organisms may be varied or mutated and that to a certain extent this is brought about by influencing the method of reproduction of the organism by cultural methods or by animal passage.

## EXPERIMENTAL

There is no doubt that when freshly isolated from the human being the organism with which we are working differs in size, morphology, and in many other ways from the end forms previously described. That it does not produce spores when living as a parasite in the body of the patient is very probable. We have tried many times to work out a system of isolation based on the assumption that spores were produced in the body of the patient, but with uniformly negative results. Variations of two fundamentally different methods have been tried.

In the first method swabs inoculated from the throat and tongue of scarlet fever patients were suspended in sterile test tubes and subjected to various temperatures for different periods. For instance, in the first stage these tubes were placed in actively boiling water for thirty minutes, the temperature of the swabs under these conditions being between 90 and 95° C. Plants made from these swabs into various media and incubated at different temperatures gave uniformly no growth. Heating at temperatures below this and varying the heating period gives unsatisfactory results.

The second method was to plant the swab directly on the various culture media and incubate these at temperatures varying from 20° to 43° C. and for different lengths of time. This growth was then washed off in sterile physiologic salt solution and subjected to sufficient heat to kill off vegetating organisms, subcultures on a suitable media being made at frequent intervals. This method also gives uniformly unsatisfactory results. This can be explained as reported in the third paper of this series, by the fact that this spore bearing coccus form, when grown in symbiosis with other organisms, fails to yield spores; also from other evidence that has been accumulated we are confident that the organism changes very considerably.

Mention was made in our third paper of a change in morphology that was noted in cultures isolated from animals dying as a result of injection of these cultures. Guinea pigs are not very susceptible to infection, but may be infected, providing the cultures are grown for some time on a fairly rich blood agar, and large doses, usually about two slants, are given. If death occurs within one day, cultures from the heart blood or peritoneal cavity usually show a large spore-bearing coccus form. From animals dying on the second to fourth day following injection, the organism isolated on autopsy is usually a peculiar small cocco-bacillus. These cultures when planted on

different sugar media are usually alkali producing. Serologically they fail to agglutinate with specific serum for the spore-bearing group, neither are they agglutinated by 16 serum. Postmortem cultures on animals dying several days after injection frequently show a hemolytic type of organism which is apparently identical with the hemolytic streptococcus of the beta type. These hemolytic organisms when carried on alkaline sugar media $P_H$ 7.8 at 37° C. gradually increase in size, and forms very similar morphologically to the spore-bearing forms may be seen, although no spores have been demonstrated and no alkali is produced in these cultures. This work will be continued and will be reported upon in detail in later papers.

We believe this to be of particular significance, due to the finding of Tunnicliff,[48, 49, 50, 51, 52] Bliss[4] and many others, who have practically demonstrated that the hemolytic streptococcus found in scarlet fever is different from the streptococci found in other diseases.

In our work all cultures are planted on various sugar media, and the reaction watched for a comparatively long period of time. In one instance a series of cultures which had been in the incubator for several weeks were examined microscopically and much to our surprise, we discovered one culture, No. 16, which had been isolated as a small cocco-bacillus, which produced alkali, had changed to the spore-producing, coccus-like form. This, in connection with our animal inoculation results, directed our attention into new channels, and since then much of our work has been confined almost entirely to a study of our pure cultures, in order that we might have some idea how to apply these results to the direct culturing of the patient, providing this peculiar organism, or group of organisms, has some etiologic connection with scarlet fever.

Culture No. 16 usually shows under the microscope what is apparently a mixture of cocci and bacilli. While we realize that perhaps many bacteriologists will say that this is not a pure culture, we must say that we think it is. We have plated this culture as high as ten times in succession and always without separating these forms. We might call attention also at this time to the statement made in many of our standard text books, that one method of obtaining a pure culture is by inoculating a susceptible animal and recovering the organism from the heart's blood. This culture is pathogenic for guinea pigs, and even after several successive animal passages, blood cultures show this same form, and as a matter of fact these purified cultures have been changed from this form to the big spore-bearing coccus forms. The same situation confronts us as confronted Mellon for example, in his studies on the cyclic or mutation changes in Corynebacterium hodgkini, etc., and which we believe he has satisfactorily answered in his later work in which single cell cultures were used. Single cell cultures have not been made by us as yet.

A point of considerable importance with regard to this culture is that when carried for some days on Löffler's media transplanting every day, forms very similar to the Klebs-Löffler bacillus are found usually predominating in the culture. These forms resemble the diphtheria organism so closely that

experienced workers may be misled by them, and call the cultures positive for diphtheria. The resemblance to Klebs-Löffler is very pronounced, and their similarity to the cultures from the discharging ears, glands, etc., following scarlet fever is a very noticeable point. This brings up the point about which bacteriologists in general are divided. Do the cultures from these discharging ears, glands, etc., contain the diphtheria bacilli or is there only a diphtheroid organism? Park inclines to the opinion that they are diphtheroids, but we cannot agree with him in all cases. Many of these discharges do, we are convinced, carry true diphtheria bacilli, but not all that are reported positive by the usual diagnostic methods are diphtheria, even though they may be reported virulent by the usual test. We would like to call attention to the advisability of making a Gram stain on these cultures, and we believe it will be admitted to be necessary. This culture, No. 16, even when

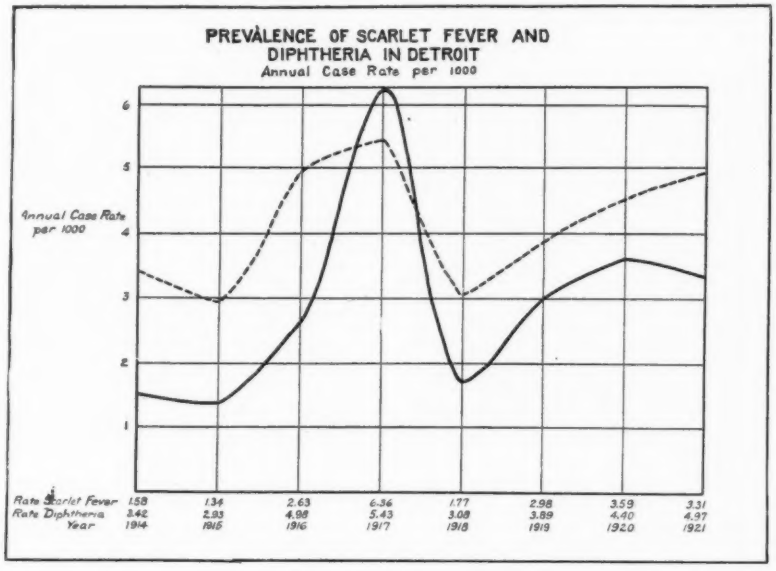
PREVALENCE OF SCARLET FEVER AND DIPHTHERIA IN DETROIT
Annual Case Rate per 1000
Annual Case Rate per 1000
6
5
4
3
2
1
Rate Scarlet Fever 1.58 1.34 2.63 6.36 1.77 2.98 3.59 3.31
Rate Diphtheria 3.42 2.93 4.98 5.43 3.08 3.89 4.40 4.37
Year 1914 1915 1916 1917 1918 1919 1920 1921

Fig. 1

very similar to the diphtheria bacillus, is frankly Gram negative. The similarity to the diphtheria bacillus is so striking, however, that it was thought advisable to see what effect, if any, ordinary diphtheria antitoxin would have on animals receiving injections of these cultures. Our animal experiment work will be reported on more in detail in a future paper in conjunction with Dr. Morgans of this staff. Certain portions of the work will be mentioned in a preliminary way here.

In this work the growth from a number of agar slants was washed off in sterile salt solution and combined so that all animals received the same dose of the same suspension. Guinea pigs of approximately 250 grams weight were used. Animals, all from the same lot, have been divided into three groups; the first, or control group, being given no antitoxin. These animals invariably died. The second group received diphtheria antitoxin at the same

time as the inoculation. Part died and part lived. The third group received antitoxin in amounts of two hundred to one thousand units, 24 hours previous to the injection of the organisms. All animals lived. This work has been repeated a great many times and with identical results. There is a possibility, of course, of there being more than one type of diphtheria bacilli. Havens has reported that he finds apparently two types of the diphtheria organisms, differing in their agglutination as well as in their toxin production. This work, however, has been vigorously disputed by Park who says that he is unable to find any marked differences in toxin production in the diphtheria organisms isolated from a large series of cases. However, we are thoroughly convinced that many times these discharges from glands and ears, etc., following scarlet fever do carry the genuine Klebs-Löffler bacilli and that diphtheria may develop in people who come in direct contact with them. In other cases, however, scarlet fever may develop.

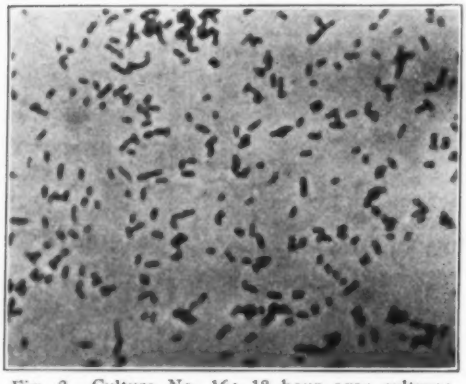

Fig. 2.—Culture No. 16; 18 hour agar culture; gram stain X1500.

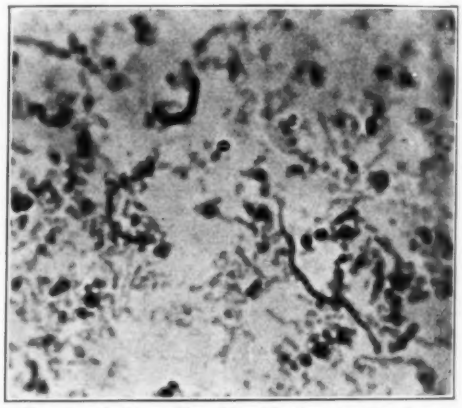

Fig. 3.—Culture No. 16; 18 day agar culture; gram stain X1500.

Cultures No. 16 when grown under conditions favorable to development of diphtheria toxin produced, after filtration through a Berkefeld filter, a poisonous substance which will kill guinea pigs. The medium that we have used in this work is similar to that described by Bunker[7] for use in the production of diphtheria toxin. We have succeeded in this way in obtaining filtrates which were poisonous for guinea pigs in doses as small as one-tenth of a cubic centimeter. The production of this poisonous substance appears to begin about the fifth day, and reaches its maximum between the seventh and ninth, and gradually disappears. Filtrate which has been preserved by the addition of one-fourth of one per cent tricresol and kept in the ice box at a temperature of 4 to 6° C. loses its poisonous property rather quickly. Young guinea pigs 100 to 150 grams in weight, seem to be much more susceptible. While we cannot say as yet that this represents the production of true toxin yet we believe that this opens a field of considerable importance in this work. To the person experienced in toxin work this looks like very weak toxin, but it is a matter of record that the first diphtheria toxin produced was much

weaker than this. Besson states that Roux and Yersin in their first work produced a toxin of which 30 c.c. were required to kill a guinea pig. We have noted a considerable difference in the reaction of different guinea pigs, though the weight may be approximately the same, to the inoculation of this poisonous product.

Epidemiologic studies show that in the northern portion of this country there is considerable evidence of a relationship between diphtheria and scarlet fever, and that the incidence curves of these two diseases run closely parallel. In Figure 1 the case rates of these two diseases for the city of Detroit have been plotted for the period from 1914-1921 inclusive, diphtheria being represented by the dotted line and scarlet fever by the solid line. With the single exception of the year 1917 the tendency of these curves to run parallel is very evident. During 1917 Detroit, in common with the northern part of the United States, suffered from a severe epidemic of scarlet fever. How-

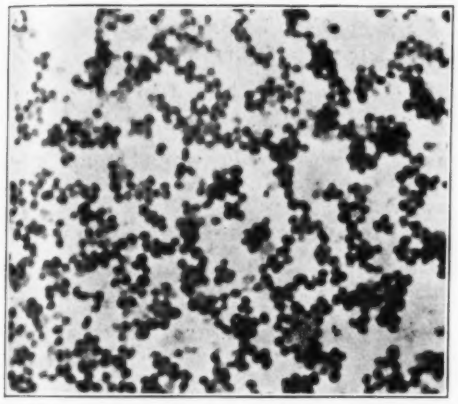

Fig. 4.—Culture No. 16. Changed to spore bearing coccus form, gram stain X1500.

Fig. 5.—Isolated from blood of guinea pig dying four days after injection of spore coccus form. Culture similar to beta haemolitic streptococcus. Culture from sucrose meat free agar six days old. Note apparent budding form. Gram stain X1500.

ever, diphtheria was very prevalent also, and although the curves cross, the general peak and valley relationship is maintained.

In view of these well recognized facts this organism which we are discussing, and which varies so widely in its morphologic and other characteristics becomes, we believe, of particular importance, and justifies the great amount of work being done on it. While this report is based very largely upon the results obtained with culture No. 16, we may say that we have a considerable series of cultures of this type which are serologically identical with culture No. 16, and which have been isolated from scarlet fever.

### THE DIAGNOSIS OF SCARLET FEVER BY LABORATORY METHODS

Owing to the tremendous amount of routine work done in this laboratory, time for research work is at a premium; however, a limited series of cases have been examined. Thirty-five cases at the Herman Kiefer Hospital were cultured by Miss R. J. Green, in charge of the Hospital laboratory, and were

turned over to us by number for examination. In most instances two swabs were made on successive days, one from the tongue and one from the tonsils. These were cultured and examined by us, using the suggestion on which this paper is based, with the following results: Of the 35, 11 were reported as positive scarlet fever, basing our report on morphology and variation of organisms in cultures. Of these 11 reported positive, 10 were scarlet fever cases and one a patient in the tuberculosis ward. Of the 24 reported negative, 3 were scarlet fever and the others not clinically scarlet fever. This makes an average report of 77 per cent correct findings in scarlet fever and of 4½ per cent incorrect findings in other than scarlet fever. It should be stated, however, that we were unable to obtain any agglutination of the culture from the tubercular case with any of our type serum.

Very favorable results have also been obtained by examination of blood cultures both from known and unknown cases. Our method has been to use 40 c.c. of the meat free serum media (reacting $P_H$ 7.5 approximately) previously described, adding to this 10 c.c. of blood (the blood being collected either in oxalate or trypsin solution) mixing and making four or five plates, and incubating at 30° C. While the number of organisms found in ten c.c. of blood is rather low many peculiar and baffling changes in morphology are observed in subcultures, particularly when alternating between meat free sucrose media and ordinary blood agar.

The many different phases of this work are under investigation in this laboratory with the assistance of various members of the staff. It must be remembered, however, that many of the variations herein reported take place very slowly and that the conditions which govern them are very largely unknown.

The writer desires to express his appreciation to Miss R. J. Green for many favors during the course of this work and to Dr. Morgans, Miss Schueren, Miss Novy, Mr. Adams, Mr. Thomas and others of this staff for their cooperation and assistance. The microphotographs are by Miss Wagner of the Detroit College of Medicine.

## SUMMARY

The organism previously reported from this laboratory as found only in scarlet fever shows several varieties, one of which is, under certain conditions, somewhat similar in its morphology to B. diphtheria and which may be a toxin producer. Diphtheria antitoxin protects against this culture, providing the antitoxin is given some time before the injection of the culture.

A preliminary report is made of results obtained by culturing patients sick with scarlet fever which we believe justifies the tentative conclusion that this variable organism is the cause of scarlet fever.

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# POSSIBLE DEFENSIVE FACTORS IN CANCER OF THE RECTUM*
## (A STUDY OF 102 CASES)

WM. CARPENTER MACCARTY, M.D., AND J. K. W. KEHRER, ROCHESTER, MINN.

*Section on Surgical Pathology, Mayo Clinic.*

IN June, 1921, one of us (MacCarty) published a series of observations on the "Relation of cellular differentiation and lymphocytic infiltration to postoperative longevity in gastric carcinoma."[1] It was then stated that the presence of these factors was associated with an 82 per cent increase in average length of postoperative life. Since that observation carcinoma of the rectum has been studied in a similar manner with the exception that such possible factors as fibrosis and hyalinization were also taken into consideration. In the series, 102 cases were studied (Charts 1 to 4). As in cases of cancer of the stomach, every patient was known to have died of local or distant recurrence following radical resection.

TABULATION OF DATA SHOWN IN CHARTS 1 TO 4.

| | |
|---|---|
| Total number of cases | 102 |
| Average length of postoperative life | 1.47 years |
| | Per cent |
| Frequency of differentiation | 86 |
| Frequency of lymphocytic infiltration | 57 |
| Frequency of fibrosis | 75 |
| Frequency of hyalinization | 2 |
| Frequency of differentiation and lymphocytic infiltration | 56 |
| Frequency of differentiation and fibrosis | 70 |
| Frequency of differentiation and hyalinization | 2 |
| Frequency of lymphocytic infiltration and fibrosis | 47 |
| Frequency of lymphocytic infiltration and hyalinization | 1 |
| Frequency of fibrosis and hyalinization | 2 |
| | Years |
| Average length of postoperative life with differentiation | 1.54 |
| Average length of postoperative life without differentiation | 1.08 |
| Average length of postoperative life with lymphocytic infiltration | 1.57 |
| Average length of postoperative life without lymphocytic infiltration | 1.31 |
| Average length of postoperative life with fibrosis | 1.53 |
| Average length of postoperative life without fibrosis | 1.29 |
| Average length of postoperative life with hyalinization | 2.33 |
| Average length of postoperative life without hyalinization | 1.44 |
| Average length of postoperative life with differentiation and lymphocytic infiltration | 1.59 |
| Average length of postoperative life without differentiation and lymphocytic infiltration | 0.71 |
| Average length of postoperative life with differentiation and fibrosis | 1.58 |
| Average length of postoperative life without differentiation and fibrosis | 1.15 |
| Average length of postoperative life with differentiation and hyalinization | 2.33 |
| Average length of postoperative life without differentiation and hyalinization | 0.61 |
| Average length of postoperative life with lymphocytic infiltration and fibrosis | 1.65 |
| Average length of postoperative life without lymphocytic infiltration and fibrosis | 1.17 |

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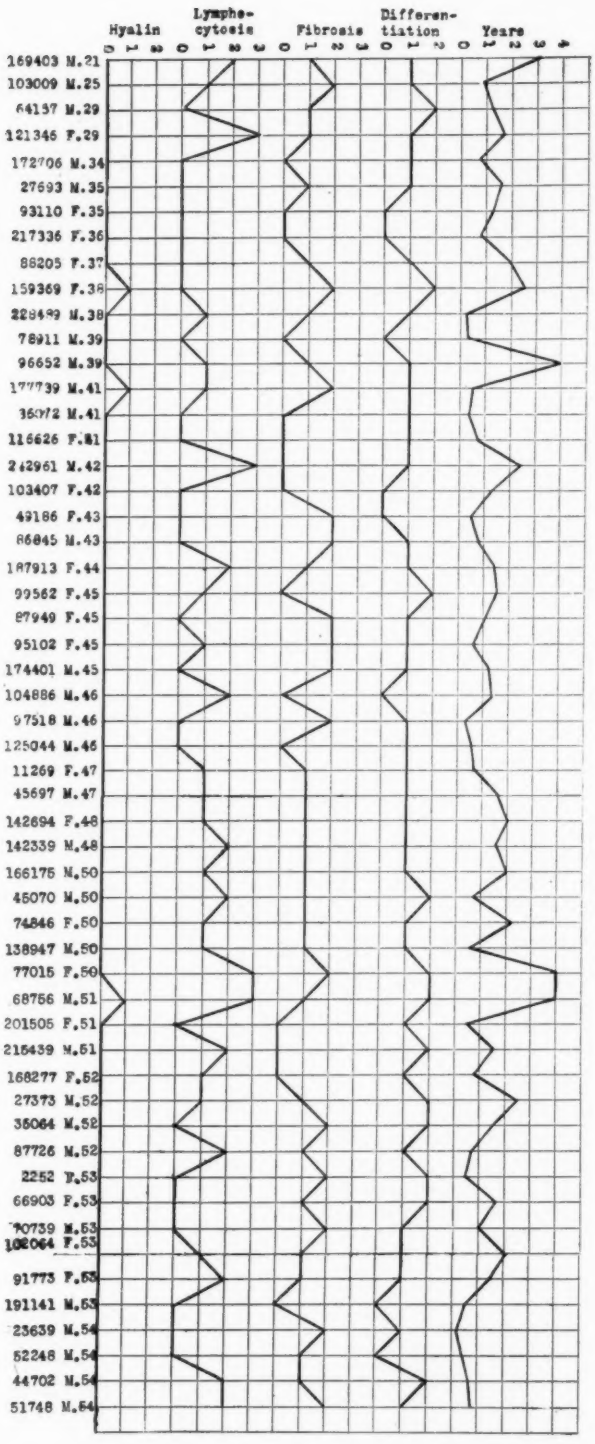
Hyalin
Lymphe-cytosis
Fibrosis
Differen-tiation
Years
169403 M.21
103009 M.25
64137 M.29
121346 F.29
172706 M.34
27693 M.35
93110 F.35
217336 F.36
88205 F.37
159369 F.38
228499 M.38
78911 M.39
96652 M.39
177739 M.41
36972 M.41
116626 F.41
242961 M.42
103407 F.42
49186 F.43
86845 M.43
187913 F.44
99562 F.45
87949 F.45
95102 F.45
174401 M.45
104886 M.46
97518 M.46
125044 M.46
11269 F.47
45697 M.47
142694 F.48
142339 M.48
166175 M.50
45070 M.50
74846 F.50
138947 M.50
77015 F.50
68756 M.51
201505 F.51
215439 M.51
168277 F.52
27373 M.52
35064 M.52
87726 M.52
2252 F.53
66903 F.53
70739 M.53
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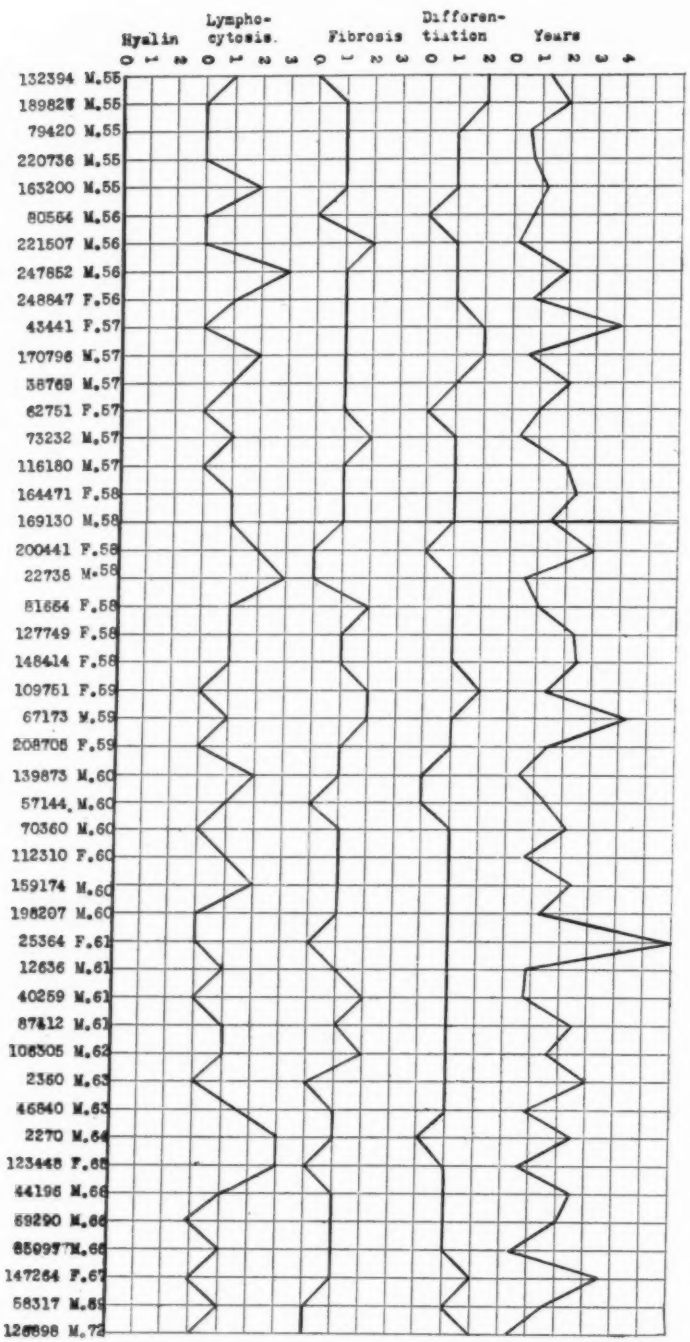
91773 F.53
191141 M.53
23639 M.54
52248 M.54
44702 M.54
51748 M.54


Hyalin
Lympho-cytosis.
Fibrosis
Differen-tiation
Years
0 1 2 3 0 1 2 3 0 1 2 3 0 1 2 3 4
132394 M.55
189828 M.55
79420 M.55
220736 M.55
163200 M.55
80564 M.56
221507 M.56
247852 M.56
248847 F.56
43441 F.57
170796 M.57
38769 M.57
62751 F.57
73232 M.57
116180 M.57
164471 F.58
169130 M.58
200441 F.58
22738 M.58
81654 F.58
127749 F.58
148414 F.58
109751 F.59
67173 M.59
208705 F.59
139873 M.60
57144 M.60
70360 M.60
112310 F.60
159174 M.60
198207 M.60
25364 F.61
12636 M.61
40259 M.61
87412 M.61
106305 M.62
2360 M.63
46840 M.63
2270 M.64
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147264 F.67
58317 M.69
128698 M.72

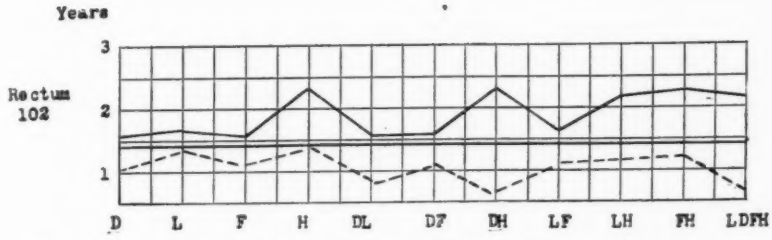


Chart 3.—Average length of postoperative life with and without factors.
Solid line, duration of life in cases with the factor. Dotted line, duration of life in cases without the factor.

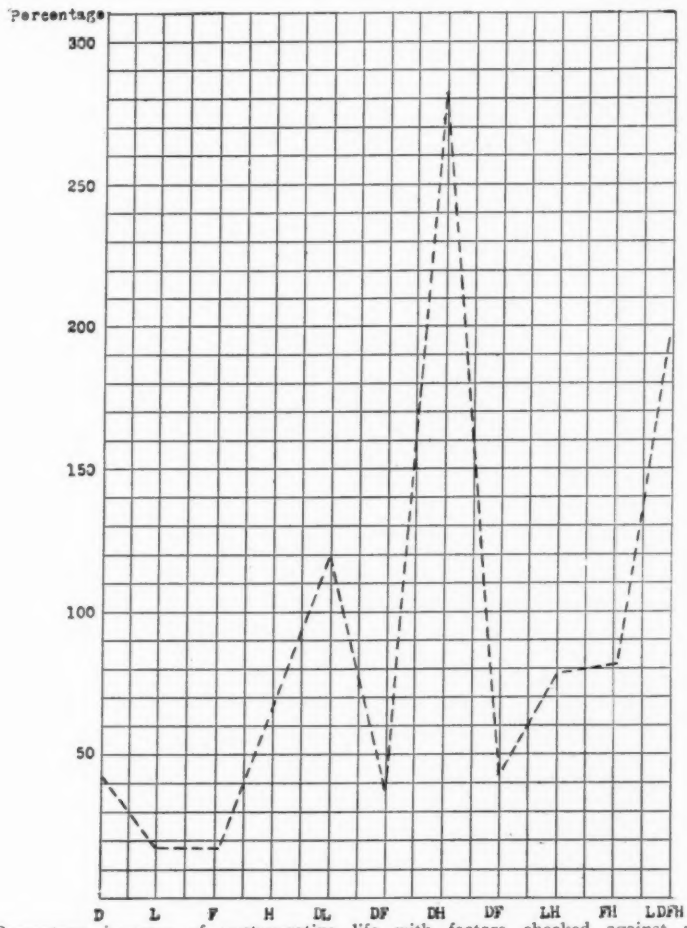


Chart 4.—Percentage increase of postoperative life with factors checked against postoperative life without factors. (Rectum.)

| | |
|---|---|
| Average length of postoperative life with lymphocytic infiltration and hyalinization | 2.25 |
| Average length of postoperative life without lymphocytic infiltration and hyalinization | 1.27 |
| Average length of postoperative life with fibrosis and hyalinization | 2.33 |
| Average length of postoperative life without fibrosis and hyalinization | 1.28 |
| Average length of postoperative life with lymphocytic infiltration, differentiation, fibrosis, and hyalinization | 2.25 |
| Average length of postoperative life without lymphocytic infiltration, differentiation, fibrosis, and hyalinization | 0.76 |

From these data certain legitimate generalizations may be made:

1. The average length of postoperative life, when the factors, lymphocytic infiltration, fibrosis, hyalinization, and differentiation, are present, is greater than when the factors are absent.

2. The average length of postoperative life, when some of the factors are present, is 40 per cent greater than the average length of life for the 102 patients, and 146 per cent greater than when none of the factors is present.

3. With all four factors present in combination the average length of postoperative life is 196 per cent greater than if none of the factors are present.

In conclusion it may be stated that from this series of observations, lymphocytic infiltration, cellular differentiation, fibrosis, and hyalinization seem to play a part in prolonging the postoperative length of life.

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## SOME SOCIOLOGICAL ASPECTS OF HEART DISEASE*

By Dr. S. Neuhof, New York

INCLUDING cardiovascular diseases in the term "heart disease," it is acknowledged that heart disease is widespread[2] and is responsible for a large proportion of deaths. Statistics vary but it is evident that valvular disease is prevalent even at the school age, and that cardiovascular disease is especially prevalent from the fortieth to the sixtieth years. The etiologic factors may be generically grouped as the infections, intoxications, and improper mode of living. This phase alone of the subject presents many aspects and angles. A few of the etiologic facts may perhaps be intelligently combated; others are unknown or are recognized only when the damage has been done to the circulatory apparatus; others have given rise to the most diverse, diametrically opposed opinions. I shall briefly sketch some of the practical difficulties of the problem, indicating some controllable, some debatable and some of the unknown causative agencies. We know for example that both lead poisoning and syphilis, especially the latter, can cause cardiovascular disease. Progress

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has already been made through educational campaigns in schools, factories, shops, amid lead workers, etc., which have tended to decrease the ravages of these factors. This is especially true of lead but much more educational work will certainly be necessary before syphilis can be classed as a definitely controllable factor. Improper food, especially an improperly balanced, high protein diet, may have a certain influence in causing arteriosclerosis; but in spite of some experimental evidence, further intensive, exhaustive and long-continued clinical study will be required before we have positive evidence that improper feeding alone is one of the fundamental causes of cardiovascular disease. Alcoholism can undoubtedly cause cardiac disease, especially myocarditis. As already pointed out, its rôle in this respect has been exaggerated. There are hopes that alcoholism, even if not entirely eradicated, will gradually become an increasingly minor cause of heart disease. Diphtheria was undoubtedly a frequent cause of cardiac disease and death. Fortunately, toxic cases are now infrequent, and the immediate use of diphtheria antitoxin cures even some of these. Hence diphtheria has become but a small etiologic factor of heart disease. Scarlet fever may be taken as the type of a frequent infectious cause of valvular disease. We possess no specific remedy that can lessen the probability of the poison affecting the heart; our efforts are practically limited to the usual routine hygienic and symptomatic procedures which need not be discussed here. Yet, after all, the danger to the heart in scarlet fever is but a small phase of the larger problem of communicable diseases with which preventive and epidemiologic medicine must cope. Overcrowding, sanitation, education of the native-born and foreign population represent some of the sociologic aspects of the same problem.

Rheumatism, especially the acute, articular variety, chorea and tonsillitis are undoubtedly the largest and most important causes of cardiac disease in middle-aged and young. Here, likewise, we possess no specific remedy which, so far as known, will minimize the danger of endocardial involvement. It is not necessary to enter again into the extremely debatable question of the bacterial origin of the disease. Even assuming this etiology, we have no antibactericidal remedy. We possess many drugs for the control of the rheumatic articular manifestations, but for the present we can only assume that these drugs thereby lessen the liability to rheumatic endocarditis. In general, it may be stated that otherwise doubtful endocardial murmurs take on more important significance in the presence of actual rheumatism. Attention of the public should be called to the dangers of rheumatism; they should be acquainted with such simple measures as we possess to prevent rheumatism; these consist chiefly in proper clothing and protection against rain and snow in the changeable climate of the temperate zone. It may even be necessary to suggest climactic changes in order to obviate rheumatic recurrences. It is not alone the marked cases of rheumatism but also the so-called "growing pains" of children which, in educational campaigns, should be emphasized as evidences of actual rheumatism. The connection between rheumatism and the tonsils and teeth will be discussed later.

The inflamed tonsil is the next most frequent etiologic factor of endo-

carditis. About a half dozen years ago, the tonsils were eradicated upon the slightest evidence of rheumatism or of suspicion of endocarditis. The views at present are not quite as radical. Tonsillectomy has assumed much importance since the demonstration that the tonsils are often the portals of entry for endocardial infections. Tonsils which are palpably diseased or which become frequently infected should be properly and thoroughly removed if the general condition of the patient warrants it. This statement also applies to adenoids. Even here, however, a time should be chosen when the endocarditis has reached a quiescent stage, for I have seen distinct harm, and indeed recrudescence of the disease caused by untimely operation on the tonsils. However, I hold conservative views regarding the removal of apparently healthy tonsils with the idea of preventing the further spread of endocarditis. I believe that when valvular disease is already present, the removal of small and healthy looking tonsils does not tend to prevent reinfection. This view is not invalidated by the fact that tonsils which look normal in the throat show, upon removal, various pathologic changes, both macro- and microscopically. Because of their function as filtering agents and because of their constant contact with, and exposure to, bacteria, the tonsils can scarcely ever represent normal lymphoid structures. It is still a moot question whether recrudescences of endocarditis have been prevented by the routine and radical practice of tonsillectomy in all children with valvular lesions. Thus, in one excellent report, such tonsillectomies did not affect the frequency of recurrences, nor the course of the disease.

The teeth have been found to harbor, and act as foci of infection in the production of some types of muscular and joint rheumatism, especially in the adult. There have been vigorous campaigns launched both among the laity and among physicians to eradicate the foci of infection, and thus prevent rheumatism and heart disease or, when already present, to prevent their further spread. X-ray examinations of the teeth have been brought as witnesses to show how widespread dental infection may be. It may perhaps be parenthetically, yet pertinently remarked, that dentists can only indicate which teeth are diseased as well as the type of disease. It remains for the physician to determine the correlation, if any, between the dental infection and the disease in question, and to decide upon the advisability and time of removal of the dental infection. As a matter of personal observation, I believe that diseased teeth cause endocarditis in only the rarest instances. In the very few I have seen, there was dental caries and septic osteomyelitis of the jaw with general septicemia, endocarditis and death. As the result of careful and long-continued observation, I believe that endocarditis in itself does not warrant more radical or more careful dentistry than is usually required for diseased teeth and purulent foci in otherwise normal individuals. Dentistry here, as elsewhere, should follow sane lines. As with tonsillectomy, I regard routine extraction for the prevention of endocarditis as uncalled for. Extensive extractions upon the supposition that exceedingly small pus foci frequently produce endocarditis is in my opinion unwarranted by general

clinical experience and by the negative results following such practice in patients with endocarditis.

The general subject of removal of foci of infection as a prophylactic, or a curative measure in heart disease has aroused the medical mind. It is undoubtedly true that a "focus of infection" anywhere in the body, e.g., the inflamed gall bladder and oropharynx, is a potential cause of endocarditis and is hence an etiologic factor to be reckoned with. I believe, however, that its frequency as a factor has been tremendously overstated. It is one thing to diagnose a focus of infection; it is another to state that it is the cause of cardiovascular disease, and still another to state dogmatically that its removal is going to benefit the patient. As a matter of fact, it can scarcely effect a cure, for, when the diagnosis of circulatory disease is made, the pathologic damage has been done. Hence, at best, eradication of infected foci can either bring only relief or quiescence of the disease. In some older individuals with cardiorenal disease and infected teeth, I saw no effect of any kind from the wholesale extraction of teeth. In other words, prophylaxis against cardiovascular disease or the presence of the latter calls for no more radical surgical intervention and eradication of infected foci than that focus ordinarily demands. To do otherwise is to lose one's clinical balance and to practice medicine by simple rule of thumb rather than by mellowed clinical judgment.

Aside from diet and therapy, other questions regarding the management of compensated and decompensated cases of cardiovascular disease arise. Some of the commoner of these are: Shall a patient return to work? What type of work shall he follow? Shall medication be continued and, if so, how long?

With respect to these questions, no matter what the type of lesion there are two preliminary fundamental considerations to be determined, namely: The degree of compensation, and the state of quiescence of the disease. With quiescent compensated lesions, valvular or myocardial in nature, the main restriction regarding exercise should be the kind, rather than the amount, provided always it be well within the patient's cardiac reserve power. This statement requires some modification, for the type of cardiac disease plays a rôle which requires some individual discrimination. For example, patients with tremendous hypertrophic left ventricles from aortic valvular lesions are scarcely able to maintain long-continued effort without soon encroaching upon their cardiac reserve. In general, however, it may be stated that even quiescent compensated cases should avoid all exercises which call for sudden or sharp exertion, as swimming a long distance, running and tennis playing. On the other hand, golf is an excellent form of moderate exercise. It entails the necessity of being away from business and of being out in the open for a number of hours, considerations which in themselves are very desirable. In exercises as well as in work it should be emphasized that patients should keep well within their individual limits of fatigue.

The question of occupation and vocation for patients with cardiac disease has recently received wide consideration from the lay as well as from the

medical standpoints. It is gradually being recognized that many individuals with cardiovascular disease are not thereby necessarily precluded from attempting to earn a livelihood, and that, if proper work be chosen, they may become self-sustaining members of the community. Occupations and vocations at which patients sit or stand are preferable to those which require walking or stair climbing. Positions in counting houses, clerical work, draftsmanship, light manufacturing industries, working at lathes or small machinery, watchmen, are examples of the work which these patients may safely follow; but just because of these sedentary and easy occupations, exercise out in the open, chiefly walking, should be advised. It is, I believe, a therapeutic error to attempt to avoid all circulatory strain by having these patients pass an almost muscularly inert existence, for it is only by mildly stimulating the circulation by appropriate gentle exercise that the heart and circulatory apparatus are kept at their proper individual levels of efficiency. In this respect the heart does not differ from other weakened muscles whose strength is enhanced by moderate, well-planned and individualized exercise. Mild appropriate dumb-bell exercises and other calisthenics should be advised when walking is not feasible, or as additions to the latter.

It has been found that when those with cardiac disease (so-called cardiacs), find employment, the fact that they are handicapped and hence cannot cope with other workmen in efficiency and earning power does not depress them; on the contrary, they find happiness and satisfaction in being able to earn something which will help them toward becoming independent and self-supporting. It is, on the whole, difficult for cardiacs themselves to find the proper type of employment. Hence, in the larger cities, bureaus are established for the poorer and needier, through which appropriate employment may be found. Under such circumstances, the bureau head seeks information from the medical chief of the cardiac clinic and from the social service department in order to determine the functional capacity, the state and type of cardiac lesion, and the home surroundings of the individual. In this manner, some individuals have been given suitable employment and made earning members of the community. In a recent report on employment for the cardiac by I. M. Duggan,[1] many diversified types of employment such as watchman, telephone operators, electricians, jewelers, elevator men, seamstresses and clerks were listed.

Placement for cardiacs has also this important medical aspect, that in some instances it may save patients from attacks of decompensation which are due to cardiac overstrain from improper and too laborious employment. To that degree, it will decrease hospital admissions from these causes, and save hospital beds and money for other patients.

Cardiac clinics are now established in some of the larger cities, not only for group treatment of cardiac disease but also to bring home to the physicians and laity the importance and size of the problem involved. The cardiac clinic must not only be thoroughly organized from the medical standpoint, but other activities are necessary and must be organized in order to carry out important sociologic work. These will be described first, the clinic itself, last.

There is the social service department, an all-important adjuvant to the clinic. Besides routine information regarding the financial status of the patient and his family, there is correlated sociomedical work as part of its domain. If, for example, the social service head be a trained nurse, she can readily control the group exercises of the cardiac, especially of the children; her medical knowledge is sufficient for her to observe beginning dyspnea, rapid or overforceful heart action, etc., in those taking part in graded exercises and games. Follow-up work by means of which home conditions are studied, parents and relatives given proper information regarding ventilation, sanitation, clothing, food and exercise, especially stair-climbing, is also part of the work of this department. It is the most important link between the clinic and the home; without it, the clinician has no way of determining whether treatment and advice given by him can or will be carried out. If one is dealing with a child, the question of schooling and, with it, the advisability of special school classes must be considered; if with an adult, the problem of proper employment must be carefully discussed. These are but two instances of the vast number of questions upon which the work of the social department touches. When carried out in a kindly, unobtrusive way, this department tends to harmoniously bind the medical and equally important social problems of the cardiac. In large cardiac clinics,[3] more active educational work may be undertaken through talks given by medical and social attendants to mothers of cardiac children. These should, of course, be simple and touch upon sanitation, ventilation, proper diet, rest, exercise, the hopeful aspect of many of the cases, the importance of operative treatment of the tonsils and teeth in appropriate cases, etc.

In well equipped hospitals under whose auspices cardiac clinics are managed, there should exist helpful cooperation. Where possible, frequent consultations between cardiac and correlated departments should be the rule in order to gain proper viewpoints and well balanced judgment regarding the necessity and type of operative treatment to be carried out. Unless unavoidable because of crowded conditions, mere routine sending of patients to nose, throat, and dental clinics is inadvisable.

Where the proper hospital facilities are at hand, x-plates or fluoroscopic examination of the chest should be made in order to have data regarding the size as well as the activity of the heart. Electrocardiograms are also of value, especially in fixing the types of arrhythmias and aiding in the diagnosis of congenital lesions.

In order to obviate the necessity for the cardiac child going home for lunch or climbing stairs at school, or even visiting the cardiac clinic, special cardiac classes are estabished in schools, lunches are supplied, rest periods regulated, and graded exercise and games carried out. When such special classes are not available, cardiac classes can be organized in health centers or settlement houses; under such circumstances a special teacher for educational purposes and a proper nurse are required. The physician can visit and examine the children in their classrooms and give such directions and instructions as he finds necessary.

All these correlated articles are naturally predicated upon the proper formation of the cardiac clinic itself. A concerted attempt among cardiac clinics has been made to standardize and simplify the classification of cardiac disease. The following has been finally adopted:

Class I—Organic (able to carry on habitual physical activity)
Class II—Organic (able to carry on diminished physical activity)
A. Slightly diminished
B. Greatly diminished
Class III—Organic (unequal to any physical activity)
Class IV—"Possible" heart disease
(Doubtful murmurs: mainly accidental, possibly organic)
Class V—Potential (predisposing history).

One may perhaps cavil at the advisability of some of these groupings. For example, "potential" cardiacs—chiefly children that have had rheumatism, chorea and frequent tonsillar attacks—should undoubtedly be carefully examined from the cardiac point of view, but I fear that overlapping of function can scarcely be avoided if "potential cardiacs" are to be regarded as actual "cardiacs." A simple grouping of organic heart disease might perhaps be that of the quiescent, the mild and the severe insufficiency cases. But for the sake of uniformity among cardiac clinics, the first mentioned classification should be followed as adopted. Once graded, it becomes a comparatively easy task to carry out appropriate treatment. Thus Class III, the severely decompensated, are not ambulatory cases. They belong at home or in the hospital. When again beginning to compensate, the question of active and passive motion, and the time for being allowed out of bed must be decided. The first class also, those with physical signs but no symptoms, are readily disposed of. Children and adults may follow the usual conservative daily routine of normal individuals. They may practice mild sports and games; but they should eschew violent sports such as football or participation in any competitive games. Laborers should, wherever possible, choose a type of work that does not require constant extreme muscular exertion. One can only generalize by stating that all patients with organic cardiovascular disease should exercise, work or play well within their individual cardiac tolerance. I believe this warning justified in spite of the well-known fact that cardiacs are found among laborers and athletes who have never shown any circulatory symptoms. In general, those with markedly hypertrophied hearts (usually aortic lesions) should be more conservatively advised regarding the type of sports and games and such daily routine as stair- and hill-climbing. Next in the scale of conservatism are cases of mitral stenosis. One may be least conservative in those of this group (first class) which have quiescent mitral regurgitant lesion.

Class II, the mild insufficiency group, is that which usually constitutes the majority of the ambulatory patients. They likewise offer the best opportunity for improvement by means of graded games and exercises. I refer here to children alone: adults of this group, if sufficiently improved, should seek suitable employment.

Exercise among children is best achieved by graded games and drills. Games that in addition give proper posture are of special advantage. Exercises should be supervised by a doctor, trained nurse or specially instructed social worker. Various types of drills—all rhythmic—may be devised. The simplest are those in which, at the beginning, all may take part. In general, they correspond to mild setting-up exercises, leaving out sharp flexing of the abdomen. Upward and lateral movements of the arms, clapping hands above the head and in front of the body, lateral and forward, lateral and slight backward bending of the body on the trunk, bending of the knees and hips are some of the usual types of drill exercises. Military tramping and shuffling steps, marking time and other exercises will readily suggest themselves.

More active games may be devised for those who have shown no circulatory strain as a result of milder exercises. Breathlessness, prolonged tachycardia, overforceful and violent heart action, drawn and tired features are some of the more obvious, readily discernible evidences of such overstrain. Such games as hopping, pitching ball, rhythmic dancing, etc., are suggested as more active games in the better compensated cases.

An excellent adjuvant to cardiacs is some form of convalescent country home. A sojourn in the country is especially advisable for those who have recently recovered from endocarditis reinfections, or who look pale, tired and undernourished. In the latter, overfeeding should be attempted. To bring these patients to normal weight is perhaps one means of strengthening the entire organism against the inroads of infection. The home should not be too far from the city so that the patients can be occasionally visited by relatives, thus forestalling nostalgia. Convalescent homes are especially appropriate for those with fair exercise tolerance.

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# ROULEAUX FORMATION OF RED CELLS IN VARIOUS TYPES OF DISEASE*

By David Brewster Swift, B.S., Boston, Mass.

## INTRODUCTION

In immunologic work complement is usually regarded as one of the most labile constituents of blood. There are, however, two other characteristics of normal human blood which are very unstable, namely, the properties of the serum and the red corpuscles which are involved in the formation of rouleaux. Under the conditions of the following experiments, normal serum completely loses its power of producing rouleaux formation of red cells in about 24 hours at room temperature, a period of time which corresponds in a rough way to the length of time required for the inactivation of complement under similar conditions. The red cells, however, are far more sensitive and after standing for a few hours (6 to 9) at room temperature they completely lose their property of being grouped into rouleaux. In view of this striking alteration in the red cells under slight changes of environment *in vitro* it seemed desirable to determine the effect of various types of disease on rouleaux formation.

## NORMAL BLOOD

*Technic.*—In freshly shed blood of man, it has long been known that the red cells are frequently grouped into rouleaux. The physico-chemical mechanism of this arrangement of cells into rouleaux is unknown. In studying this phenomenon it is advantageous to employ a technic which permits experimental control of the concentration of cells and the quantity of serum employed. In the following experiments constant and definite results were obtained with normal blood by taking advantage of the experience of Sellards[1] who found that the heating of normal serum for a short time greatly enhances its power of producing rouleaux. Equal parts of normal serum and a 10 per cent suspension of cells in saline form practically no rouleaux. After slight heating for a short time, the serum produces intense rouleaux but this property is readily lost on slight dilution. Furthermore the concentration of cells is very important since the ease of formation of rouleaux increases in direct proportion to the concentration of the suspension of cells. This is shown in the following experiment in which equal parts of normal serum

*From the Department of Tropical Medicine of the Harvard Medical School and the Collis P. Huntington Memorial Hospital of Harvard University, Boston.

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heated for 30 minutes at 60° C. were mixed with cellular suspensions of varying concentrations.

| | Strength of Cell Suspension | | | | | |
|---|---|---|---|---|---|---|
| Rouleaux formation | ½% none | 1% none | 2% partial | 5% complete | 10% complete | 50% complete |

The following routine was adopted. A few drops of blood were collected from the finger or ear in a Wright tube and centrifugalized. The serum was pipetted off and heated in a sealed glass tube for 30 minutes at 60° C. For the red cells, a little blood was collected from the ear in 0.5 per cent sodium citrate in physiologic saline contained in a graduated 15 c.c. centrifuge tube. The cells were thrown down and washed once with saline. As a routine, a 20 per cent suspension of cells was used; any departures from this concentration are noted in the text. The precaution was taken to collect normal blood at the same time as the patient's and to test the specimens promptly after collection on account of the rapid deterioration which occurs at room temperature. Equal parts of heated serum and cellular suspension were measured by a capillary pipette and mixed on a slide. From this mixture an ordinary hanging drop was prepared and kept at room temperature. When patients' cells were tested against normal heated serum the isoagglutination which occurred did not interfere in any way with the microscopic examination for rouleaux. Observations were made at the end of five minutes, thirty minutes, and one hour. Rouleaux formation often took place in 5 minutes and, as a rule, it did not increase after 30 minutes. An arbitrary method to describe the degree of rouleaux formation in the preparation has been used, namely, none, slight, partial, and complete; the last term indicating intense formation of cells in long chains with but few cells lying free in the field.

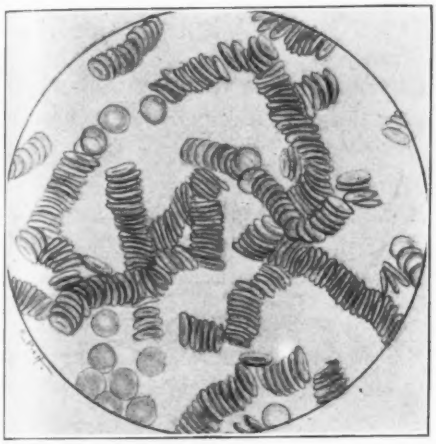

Fig. 1.—Normal cells, 20 per cent suspension, and an equal part of normal heated serum.

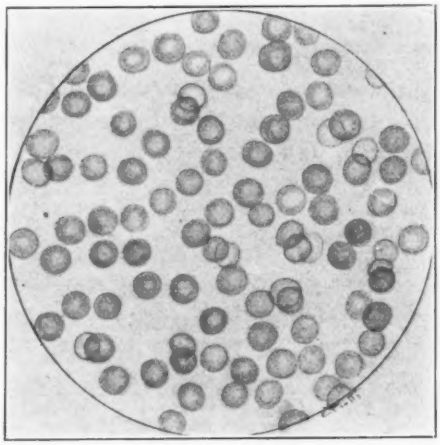

Fig. 2.—Cells from a case of Hodgkin's disease, 20 per cent suspension, and an equal part of normal heated serum. (Case No. 15.)
This figure also illustrates accurately the appearance of normal cells mixed with *unheated* normal serum.

On each day when a specimen of patient's blood was tested a parallel

examination of normal blood was made for the purpose of controlling the technic. These controls are not always recorded in the text since they were invariably performed and always gave constantly uniform results. It will be seen that this technic is designed to detect primarily those cases in which rouleaux formation is decreased rather than increased.

*Pathologic Conditions.*—Isolated observations are found in the literature on the effect of various diseases on rouleaux formation but no systematic investigation of the subject has been conducted. These scattered observations will not be reviewed here since the technic of these observers differs so much that a comparison of results is not feasible.

*Hodgkin's Disease.*—Particular attention was given to Hodgkin's disease and the following four preparations were made in the examination of each case, viz.:

1. Heated patient's serum + patient's cells
2. Heated normal " + " "
3. Heated patient's " + normal "
4. Heated normal " + " "

The patient's serum invariably behaved like normal serum showing that where rouleaux formation failed, the deficiency lay in the patient's cells. The results are shown in Table I.

TABLE I

ROULEAUX FORMATION IN CASES OF HODGKIN'S DISEASE

| SERIAL NO. | PATIENT'S CELLS 20% | 10% |
|---|---|---|
| 1 | None | — — |
| (one day later) | " | — — |
| 2 | " | — — |
| (one day later) | " | — — |
| 3 | Partial | — — |
| 4 | Complete | Complete |
| 5 | " | Partial |
| 6 | None | None |
| 7 | Slight | None |
| 8 | None | None |
| (2 days later) | " | " |
| 9 | Complete | — — |
| 10 | Partial | Partial |
| (1 month later) | " | Slight |
| 11 | None | None |
| (1 month later) | " | " |
| 12 | Complete | Complete |
| (11 days later) | " | " |
| 13 | " | " |
| 14 | None | None |
| 15 | " | " |
| (2 months later) | " | " |
| 16 | Partial | Complete |
| 17 | Slight | None |
| *18 | Complete | Complete |
| *19 | None | None |
| *20 | Partial | None |

*Suspected cases of Hodgkin's disease. No diagnosis made.

From this group of 17 cases in which a diagnosis of Hodgkin's had been made, there were 7 in which rouleaux formation was practically absent. These patients were under treatment with radium at the Huntington Hospital. They have not been studied sufficiently over a long enough period of time to consider the question of whether there is any relationship between the course of the disease and rouleaux formation.

*Leukemias.*—The blood of six cases of myelogenous leukemia was tested and in two, complete rouleaux formation occurred. The results are given in Table II.

TABLE II

ROULEAUX FORMATION IN MYELOGENOUS LEUKEMIA

| SERIAL NO. | PATIENT'S CELLS 20% | | NORMAL CELLS 20% | |
|---|---|---|---|---|
| | HEATED PATIENT'S SERUM | HEATED NORMAL SERUM | HEATED PATIENT'S SERUM | HEATED NORMAL SERUM |
| 1 | Complete | Complete | Complete | Complete |
| (1 day later) | " | " | " | " |
| 2 | None | None | " | " |
| (1 day later) | " | " | " | " |
| 3 | " | " | " | " |
| (2 days later) | " | Slight | " | " |
| 4 | — | None | — | " |
| 5 | — | Complete | — | " |
| (1 day later) | — | " | — | " |
| 6 | — | Partial | — | " |

In lymphatic leukemia the results were somewhat similar to those obtained with the myelogenous type. The results are shown in Table III. These four cases of leukemia had been under treatment with radium for varying periods of time, as had the case of myelogenous leukemia and Hodgkin's disease.

TABLE III

ROULEAUX FORMATION IN LYMPHATIC LEUKEMIA

| SERIAL NO. | PATIENT CELLS 20% | | NORMAL CELLS 20% | |
|---|---|---|---|---|
| | HEATED PATIENT'S SERUM | HEATED NORMAL SERUM | HEATED PATIENT'S SERUM | HEATED NORMAL SERUM |
| 1 | Partial | Partial | Complete | Complete |
| (1 day later) | Slight | " | " | " |
| (2 months later) | — | Complete | — | " |
| (1 week later) | — | " | — | " |
| 2 | Partial | Partial | Complete | " |
| (1 day later) | " | " | " | " |
| 3 | " | " | " | " |
| (1 month later) | " | " | " | " |
| 4 | None | None | " | " |
| (1 day later) | Complete | Complete | " | " |
| (1 day later | — | Partial | — | " |

*Malignant Disease.*—In contrast to the abnormal rouleaux formation obtained in some cases of leukemia, and in Hodgkin's disease, entirely typical

rouleaux formation was obtained in eleven cases of advanced carcinomatosis and three cases of melanotic sarcoma.

*Pernicious Anemia.*—Wiltshire[2] makes the statement that rouleaux formation is defective in pernicious anemia. Five cases of typical pernicious anemia were tested and all gave entirely normal results.

*Nephritis.*—Five cases of advanced chronic nephritis were tested and in each instance normal results were obtained both with the patient's serum and with the patient's cells.

*Infectious Diseases.*—No abnormal results were obtained in any of the twenty-one cases of infectious diseases which were tested during the acute stage. The following list comprises the cases which were examined: Ten of pulmonary tuberculosis, 4 of lobar pneumonia, 3 of diphtheria, 2 of measles, one of whooping cough, and one of ulcerative endocarditis.

## SUMMARY

In the study of rouleaux formation in disease, emphasis is placed upon the necessity of a technic which permits quantitative measurements by varying the dilution of the serum and the cellular suspensions.

In 7 out of 17 cases of Hodgkin's disease rouleaux formation was completely lacking under conditions in which normal red cells formed intense rouleaux. In all the cases of leukemia examined, rouleaux formation was lacking to a greater or lesser degree.

It was thought that this deficiency of the red cells from cases of Hodgkin's disease and leukemia might be due to the anemia which occurs in these diseases and that the change is not due to the anemia *per se*, is shown by the fact that the red cells of pernicious anemia formed rouleaux well.

Normal rouleaux formation always occurred in the cases of cancer and sarcoma and in the cases of infectious disease that were tested. These results were clear cut. On the contrary the results obtained in cases of leukemia and Hodgkin's disease were variable. It would seem that if there is any valuable information to be gained by testing for rouleaux formation, some finer development or adjustment of technic than that used must be established.

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# *LABORATORY METHODS*

## THE PRECIPITIN TEST IN THE DETECTION OF BACTERIUM DIPHTHERIAE*

G. H. SMITH, PH.D., AND C. E. KAUFMAN, M.D., NEW HAVEN, CONN.

VARIOUS modifications in the routine method for the bacteriologic diagnosis of diphtheria have been suggested from time to time. In all, the underlying purpose has been to reduce the time necessary for making the laboratory diagnosis, either by the utilization of a culture medium better adapted to the growth of the organism or by the application of a staining procedure which will more certainly detect *Bact. diphtheriae* when present in limited numbers or in atypical form. None of these methods have replaced the older technic since the difficulties inherent in bacteriologic diagnosis could not be overcome entirely by any of the modifications suggested.

It seemed possible that the period necessary for making a diagnosis might be shortened by resorting to serologic methods. Furthermore, if the serologic test possessed specificity the difficult matter of differentiating between *Bact. diphtheriae* and the pseudodiphtheria forms, particularly in young growths, might also be avoided. Earlier studies in pneumonia[1, 2] and more recent work in gonorrhea[3] have shown that fairly satisfactory and expeditious results can be secured by the precipitin test. An attempt has been made to adapt this test to the detection of *Bact. diphtheriae.*

The value of such a method depends upon the specificity of the reaction as well as upon the delicacy of the test. By means of agglutination and agglutinin absorption tests Langer,[4] Przewoski,[5] Lipstein,[6] van Riemsdijk,[7] Mason,[8] and others have shown that antibacterial diphtheritic sera react with diphtheria antigens in a specific manner and show no cross reactions with pseudodiphtheria strains. The work of Durand[9, 10] would indicate that a grouping of diphtheria strains into antigenic groups occurs when monovalent sera are employed in dilutions above the nonspecific zone. It thus appeared that any reactions secured might possess the requisite degree of specificity.

The technic for preparing the reagents;—precipitin and precipitinogen—the procedure for conducting the test; and the results which have thus far been secured are described below.

The precipitating sera have been derived from two sources, (*a*) rabbits immunized with single strains of *Bact. diphtheriae* and (*b*) antidiphtheritic

*From the Department of Pathology and Bacteriology, Yale University School of Medicine, New Haven, Conn.

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horse serum.* Rabbits were immunized with freshly isolated strains from clinical cases of diphtheria and the organisms used were of established virulence and presented all the characteristic cultural requirements of *Bact. diphtheriae*. In addition, for purposes of controlling the tests, immune sera were produced with diphtheroid organisms. Not all rabbits so treated have yielded sera of suitable precipitating properties, yet with certain animals sera of high titre have been secured. The difficulties associated with the production of such sera led us to try an antidiphtheritic horse serum and the results secured with this serum were sufficiently favorable to justify us in discontinuing the immunization of rabbits.

For the preparation of antigen, extracts of *Bact. diphtheriae* were secured by breaking down the organism with an alkali hypochlorite solution. By this method antigens have been prepared not only from pure cultures of *Bact. diphtheriae* but also from mixed cultures and from nose and throat swabs taken directly from the patient. Following the observation that satisfactory antigens could be derived from swabs, these have been employed exclusively since at least twelve hours can be saved by eliminating cultural procedure. The method of extracting the antigen is essentially that of Krumwiede and Noble. It consists in introducing the swab into 0.5 to 1.0 c.c. of "Antiformin" and extracting by boiling for several minutes. The solution is cooled and neutralized in large part before the addition of indicator. This neutralization is accomplished by the addition of 3 to 5 drops of N/1 HCl, after which indicator (phenolphthalein) is added and the neutralization quickly completed by further addition of the requisite amount of acid. The end-point is approximately $P_H$ 8.3. This solution is precipitated by the addition of 5 to 10 volumes of 95 per cent alcohol, after which the precipitate, which appears immediately or after standing for a short time, is collected by centrifugation. Precipitation may be hastened and the volume increased by heating immediately after the addition of the alcohol. The precipitate is taken up, with boiling, in 1 to 2 c.c. of physiologic saline. Usually such extracts are practically water-clear; if not, centrifugation will remove the turbidity. Occasionally an extract will again become alkaline upon the addition of the salt solution or during final heating, and under such circumstances the reaction should be restored with acid. The antigens should be prepared in as concentrated a form as possible; in some instances where questionable reactions have been secured a concentration of precipitinogen has been effected by further boiling.

It may be added that swabs secured from the nose and throat have remained at room temperature for as long as 60 days and have still been found suited to the extraction of antigen, although the yield or activity of the antigen so obtained has appeared to be diminished.

The test itself consists simply in layering these extracts upon the precipitating sera. In order to conserve sera and to conduct the reaction with a minimal amount of concentrated antigen the tests are set up in small tubes of about 4 mm. diameter. As a rule the reaction appears almost immediately

*Kindly supplied by the H. K. Mulford Company.

in the form of a ring of precipitate at the point of contact of the two liquids. With the weaker antigens incubation at 37° C. for 10 to 30 minutes may facilitate precipitation.

The reactions with the antidiphtheritic serum are always controlled by combination of the antigen with a normal serum and with a diphtheroid immune serum. The entire procedure—from the time of taking the swab from the patient to reading the results in the laboratory—can all be accomplished well within one hour. When a number of tests are to be made the procedure can be carried through on the entire series simultaneously with a considerable saving in time.

With this technic a considerable number of swabs have been tested and the results of a series of such examinations are presented in the following table:

TABLE I

| CULTURAL EXAMINATION | SEROLOGIC EXAMINATION | |
|---|---|---|
| | Positive | Per cent |
| Positive 44 | 43 | 97.7 |
| | Negative 1 | 2.3 |
| Negative 30 | Positive 8 | 26.6 |
| | Negative 22 | 73.4 |

From this table it is clear that the method outlined above will yield satisfactory results if cultural examination is accepted as the standard for comparison. When *Bact. diphtheriae* was present on the swab in sufficient numbers to yield a positive culture the precipitin test was positive in 97.7 per cent of the cases. With specimens culturally negative the precipitin test agreed in 73.4 per cent and the discrepancy between the two methods is in the direction of an excess of positive findings with the serologic method. It may be said, however, that none of these "negative culture-positive precipitin" reactions occurred in cases in which there was any reason to suspect diphtheritic infection. Moreover in several instances in which cultures and precipitin tests were made upon individual cases over a series of days it developed that although cultural examinations did not yield uniformly consistent results from day to day the precipitin tests were positive throughout the period.

The excess of positive precipitin reactions in the series giving negative cultural findings suggests that organisms may be detected by the serologic method even though they are present in numbers insufficient to give a positive growth upon media when inoculated in mixed culture. The difficulties connected with securing positive cultures when but few organisms are present have been pointed out by Wang[11] and others.

A certain number of questionable reactions may be expected. In the series upon which Table I is based one autolysate was secured which gave a precipitate with normal serum and none with an antidiphtheritic serum; and

in the case of a second autolysate positive reactions were secured with both the normal and immune sera. In this last instance dilution of the antigen caused the reaction with the normal serum to disappear while that with the antidiphtheritic serum persisted. The greatest possibilities for error in performing the test are associated with a too great acidity or alkalinity of the precipitinogen, or in failing to drive off all alcohol during the final heating. In no instance have we observed any nonspecific effects due to the presence of other organisms in association with *Bact. diphtheriae* upon the swabs, nor have we noted any quantitative differences that would suggest an error in the method such as might be associated with the antigenic grouping described by Durand.

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# A MICROMETHOD FOR NONPROTEIN NITROGEN USING TWO-TENTHS OF A CUBIC CENTIMETER OF BLOOD*

By C. M. Wilhelmj,† St. Louis, Mo.

A METHOD for the estimation of nonprotein nitrogen in which the blood can be obtained from the finger or lobe of the ear, obviously has its place in clinical routine. It is becoming more and more important for the clinician to determine at frequent intervals the effect of treatment, diet, and so forth, in the various conditions complicated by a high nonprotein nitrogen content of the blood. The method described here gives results which I believe are sufficiently accurate for clinical interpretation and the ease with which the blood is obtained makes it possible to have determinations done at short intervals without great inconvenience to the patient.

## THE METHOD

The blood is obtained from the lobe of the ear or the finger, and for this purpose a spring lancet is especially suitable since the depth of the puncture can then be accurately gauged and sufficient blood obtained by one

*Received for publication, October 28, 1921.
†From the Medical Service of the Jewish Hospital, St. Louis, Mo.

puncture. The blood is drawn into the blood pipette up to the .2 c.c. mark and immediately discharged into the tube graduated at 2.5 c.c., which contains a very small amount of finely powdered potassium oxalate. The blood pipette is then rinsed *twice* by drawing distilled water up to the .2 c.c. mark and this is added to the blood. Five per cent trichloracetic acid is then added up to the 2.5 c.c. mark, a few drops at a time, shaking after each addition. The coagulum is a chocolate brown color. The mixture should next be stoppered and allowed to stand for 20 or 30 minutes. At the end of this time it is filtered, using a filter paper just large enough to accommodate the whole mixture. One c.c. of the protein-free filtrate is transferred to the digestion tube,* and .2 c.c. of the digestion mixture added. The digestion is preferably done over a microburner, the tube being held in a test tube clamp or a band of heavy paper. The contents of the tube must be kept in constant circular motion all during the digestion in order to prevent loss of fluid by "bumping." The fluid rapidly boils down until only a small amount remains and this gradually becomes dark brown in color. Dense white fumes soon begin to fill the tube and when this occurs the rate of boiling should be slightly reduced. The color of the fluid gradually becomes lighter and finally becomes clear pale green; the boiling should be continued for about two minutes after this change occurs. Complete digestion should not take over 12 or 15 minutes. The solution is now cooled, and distilled water added, and the contents transferred to one of the *ungraduated* tubes of the aeration apparatus. The digestion tube is repeatedly rinsed with distilled water and these rinsings added to the contents of the aeration tube until it has been made up to a volume of about 8 or 10 c.c. (not over). One-half c.c. of N/10 Hydrochloric acid is put in the *graduated* aeration tube and the volume made up to 5 c.c. with distilled water. About 5 c.c. of fairly strong sulphuric acid is put in the remaining *ungraduated* tube and the apparatus connected with the filter pump as shown in the figure. Just before the air current is started, two pieces of stick sodium hydroxide about ¼ of an inch long are cut in half and added to the middle tube which contains the digested filtrate. The air current is then started and run at slow speed for the first five minutes and rapidly for 20 or 25 minutes longer, depending upon the rate of the air current used.* When the aeration is complete, 1 c.c. of the Nessler-Winkler solution is diluted with an equal volume of water, and 1 c.c. of this diluted solution added to the contents of the graduated aeration tube, and the volume made up to the 8 c.c. mark with distilled water. The resulting color is a reddish brown but should be absolutely crystal-clear since even a faint turbidity makes it unfit for comparison. The Nesslerized solution is now thoroughly mixed and transferred to the cup of either a Kober or Bock-Benedict colorimeter and compared with a standard solution. I have used

*The digestion tube may be lightly graduated at 1 c.c. and the filtrate run directly from the funnel into this.

*With a sufficiently rapid air current this time may be cut down to 10 minutes. The length of time required with a given apparatus may be determined by adding Nessler solution to the middle tube, which should remain colorless if all of the ammonia has been driven off.

both of these colorimeters with this method and find the 8 c.c. of solution to be sufficient to make the comparison.

The standard is prepared by adding 1.5 c.c. of standard nitrogen solution to 75 c.c. of distilled water, in a 100 c.c. graduate; to this 5 c.c. of undiluted Nessler-Winkler solution is added, and the contents made up to 100 c.c. with distilled water. The comparison is usually best made with the standard cup set at 20.

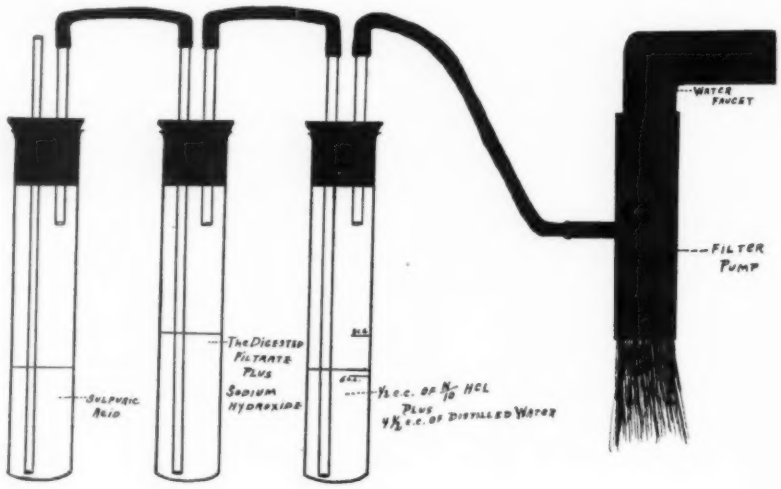
WATER FAUCET
FILTER PUMP
SULPHURIC ACID
THE DIGESTED FILTRATE PLUS SODIUM HYDROXIDE
½ c.c. OF N/10 HCL PLUS 4½ c.c. OF DISTILLED WATER

Fig. 1.

*Calculation:*

Since the equivalent of .08 c.c. of blood is compared with the standard at a volume of 8 c.c. the equation becomes:

$$\frac{\text{Reading of the standard}}{\text{Reading of the unknown}} \times \frac{30}{1} = \text{mg. of nitrogen per 100 c.c. of blood.}$$

*Example:*

Standard 20

Unknown 15

$$\frac{20}{15} \times \frac{30}{1} = 40 \text{ mg.}$$

*Reagents Required**

1. A 5 per cent solution of trichloracetic acid.
2. Digestion mixture consisting of: 1.5 c.c. of 10 per cent copper sulphate solution; 15 c.c. of distilled water; 15 c.c. of concentrated sulphuric acid; 1.5 grams of potassium sulphate. This mixture is thoroughly shaken and kept in a well-stoppered bottle.
3. Stick sodium hydroxide.
4. N/10 hydrochloric acid.
5. Nessler-Winkler solution†

| | |
|---|---|
| Mercuric iodide | 10 gm. |
| Potassium iodide | 5 gm. |
| Sodium hydroxide | 20 gm. |
| Water | 100 c.c. |

*Great care should be taken to secure nitrogen-free reagents.

†Directions from Hawk's Physiological Chemistry.

The mercuric iodide is rubbed up in a small porcelain mortar with water, and washed into a flask and the potassium iodide added. The sodium hydroxide is then dissolved in the remaining water and the cooled solution added to the above mixture. The solution cleared by standing is preserved in a dark bottle.

6. Standard nitrogen solution.

Prepared by dissolving 0.764 grams of pure ammonium chloride in 1000 c.c. of distilled water. One c.c. contains .2 of a mg. of nitrogen.

7. Powdered potassium oxalate.

*Apparatus Required:*

1. A blood pipette graduated at .2 c.c. These can be obtained from Eimer & Amend, New York, who supply a similar pipette with the Epstein Microsaccharimeter.

2. A small short tube accurately graduated at 2.5 c.c.

3. A small, long-stemmed funnel.

4. A digestion tube of thin hard glass, preferably about 1.1 cm. in diameter and not over 9.5 cm. in length.

5. Aeration apparatus consisting of: A filter pump; 3 test tubes, 1.8 cm. in diameter and 15.5 cm. in length, one of the tubes to be accurately graduated at 5 and 8 c.c. Three two-hole rubber stoppers to fit the above tubes, with holes to fit the glass tubing. Glass tubing about 3 mm. in diameter. Rubber tubing to connect the glass tubing. The aeration apparatus is connected as shown in Fig. 1.

TABLE I

| CASE | MICROMETHOD | FOLIN METHOD | DIFFERENCE |
|---|---|---|---|
| 1 | | | |
| Cardio-Nephritic | (1) 41.4 | 40 | +1.4 |
| | (2) 40 | | |
| 2 | | | |
| Acidosis? | (1) 42.1 | 40 | +2.1 |
| | (2) 45.4 | | +5.4 |
| 3 | (1) 30 | 35.1 | −5.1 |
| Healthy | (2) 35 | | − .1 |
| 4 | | | |
| Skin condition | 38.6 | 38.2 | + .4 |
| 5 | | | |
| Septic pneumonia | 41.3 | 43.1 | −1.8 |
| 6 | | | |
| Inguinal adenitis | (1) 40 | 34.5 | +5.5 |
| | (2) 40 | | +5.5 |
| 7 | | | |
| Luetic stricture of rectum | 40 | 37.5 | +2.5 |
| 8 | | | |
| Abortion | (1) 33.3 | 31.6 | +1.7 |
| | (2) 33.8 | | +2.2 |
| 9 | | | |
| Acute nephritis | 35.2 | 32.4 | +2.8 |
| 10 | | | |
| Uremia | 69.2 | 66.4 | +2.8 |
| 11 | | | |
| Healthy | 38.7 | 33.6 | +5.1 |
| 12 | | | |
| Nephritis | 40 | 38.7 | +1.3 |
| 13 | | | |
| | 42.8 | 41.4 | +1.4 |
| 14 | | | |
| Healthy | (1) 29.6 | — | — |
| | (2) 30 | | |

### DISCUSSION

The method of Folin[1] is without doubt the most accurate colorimetric procedure for the estimation of nonprotein nitrogen and hence was selected as a standard of comparison for this method. Table I shows the comparative results, with duplicate determination by the micromethod in some cases. Here it is seen that in a total of 16 readings the micromethod gave higher results than the Folin in 13 and lower results in 3. The greatest difference is plus 5.5 mg.

Greenwald[2] who first advocated the use of trichloracetic acid as a protein precipitant, advised that the protein-free filtrate be shaken with nitrogen-free kaolin and again filtered. He stated that when this step was omitted the results were about 2 mg. higher than when it was used, and since this step has been omitted in the above method this, no doubt, accounts for the almost uniformly higher readings. However, a difference of 5 mg. is hardly sufficient in clinical interpretation to lessen the usefulness of this method. This method is intended for clinical diagnostic purposes, not for research, where a higher degree of accuracy is desirable.

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## THE CELLULAR ELEMENTS OF THE CEREBROSPINAL FLUID*

By A. Levinson, M.D., Chicago, Ill.

THE cellular elements of the cerebrospinal fluid occupy an important place among the many unsettled problems of this body fluid. The origin of the cells in the normal fluid is no more settled than is the origin of the fluid itself. Nor are the types of abnormal cells in various diseases a matter of common knowledge. Yet a close study of cerebrospinal fluid does show certain types of cells in certain diseases of the meninges or of the brain. Furthermore, the cells may be used as an aid in diagnosis, and occasionally also as a guide in treatment. It is to the various types of cells in the fluid that I would like to call attention here.

### TECHNIC

Before discussing the different types of cells, I would like to call attention to the fact that not every stain used for blood cells brings out the cells in the cerebrospinal fluid vividly. Wright stain, for instance, does not bring out the structure of the cells in detail. Nor does methyl-violet or carbolthyonin. In my work I found methyl-blue the best stain for the cellu-

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lar structures of the cerebrospinal fluid. Next to methyl-blue, I found the Gram stain which is counterstained with methyl-blue most useful. Safranin makes an excellent counterstain from the standpoint of the bacteria. It does not, however, always bring out the details of the cells. The same is true with Bismark brown. Gram stain which is counterstained with methyl-blue would answer both purposes, in showing the type of bacteria and the cells. Some workers, however, cannot distinguish between violet and blue. It is therefore best to use the Gram-safranin stain for the bacteria and plain methyl-blue for the cells. Both stains can be made in a few minutes as against staining with Wright stain which is time consuming.

Another important point in the technic is to examine the cells in the fluid soon after the fluid has been withdrawn from the body, otherwise the cells degenerate.

### NORMAL FLUID

It is agreed upon that normal fluid contains only four to six cells per c.m. and that all of them are small lymphocytes. My belief is that the cells in normal fluid are derived from the blood, the chorioid barrier permitting only a minimum number of cells to pass through its walls, just as it permits small amounts of the chemical constituents of the blood to pass through into the fluid. This, however, is merely an opinion. The origin of the cells in normal fluid has not been settled as yet.

### PATHOLOGIC FLUID

Any infection of the meninges gives rise to an increased number of cells in the cerebrospinal fluid. Irritation of the meninges or of the brain may also cause an increase of cells. The number of cells, however, is not nearly as great in irritative conditions as in destructive processes. In meningism for instance, although the amount of fluid is increased the cells may not be increased in number, and when they are increased, they are all lymphocytes in type. In tuberculous meningitis and in certain stages of poliomyelitis, the cells are mainly lymphocytes. On the other hand, the cells in all suppurative meningitides are polymorphonuclear in type. In addition to the polymorphonuclear cells, however, there are also many endothelial cells. This type of cell is typically predominant in meningococcus meningitis. The same is true with the phagocytes which are present in large numbers in meningococcus meningitis (Figs. 1 and 2). In tumor of the brain, large lymphocytes are often present.

A description of the cells found in cerebrospinal fluid is given below in order of their frequency, based on my own work and on the work of other authors.

1. *Small lymphocyte* (*small mononuclear leucocyte*).—This type of cell, which is present in small numbers (1 to 6) in normal fluid and which is present in large numbers in tuberculous meningitis, poliomyelitis and luetic infections of the meninges, is the size of a red cell and contains a deeply staining nucleus, which fills nearly the whole cell. The small amount of remaining protoplasm takes the Wright, Unna-Pappenheim, or methyl-blue stain less deeply than the nucleus and contains no granules.

2. *Large lymphocyte (large mononuclear leucocyte).*—This cell which is occasionally present in normal fluid is present in large numbers in chronic inflammatory conditions of the meninges such as luetic affections of the meninges and in brain tumor. The cell is twice the size of a red cell and contains a round, less frequently an oval, nucleus which fills the large part of the cell and which stains deeply.

3. *Polymorphonuclear leucocyte.*—This type of cell is found in great numbers in the fluid of all septic processes of the meninges, in meningeal hemorrhage and at times also in brain abscess. The cell is one and a half to two times the size of a red blood cell. The nucleus is subdivided into 2, 3, or 4

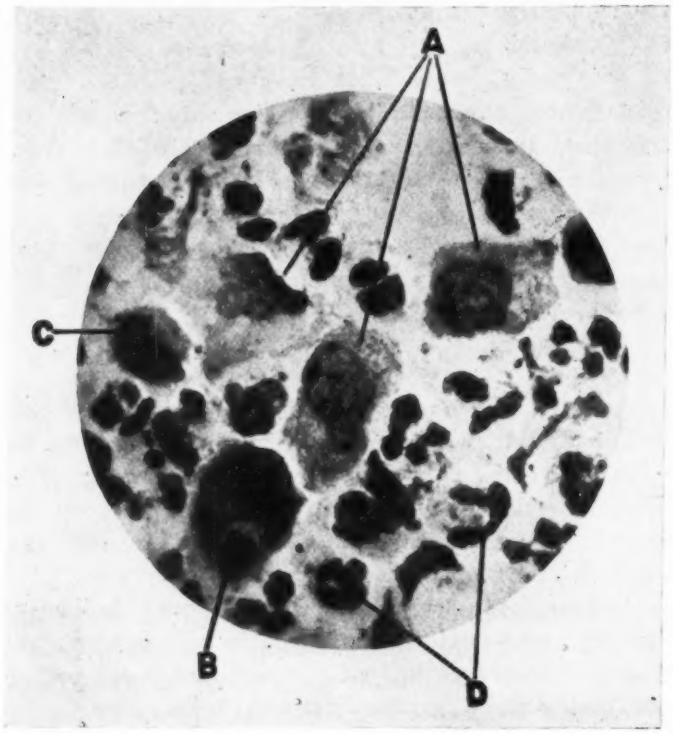


Fig. 1.—Photomicrograph showing types of cells in pathologic cerebrospinal fluid. X1000. A, endothelial cells. B, endothelial cell which has engulfed a polymorphonuclear leucocyte. C, large mononuclear cell. D, polymorphonuclear leucocytes.

lobules and the protoplasm contains many granules, the granules being either small and neutrophilic in character or large and eosinophilic. Most of the polymorphonuclear leucocytes found in the fluid are neutrophilic, eosinophilic leucocytes being found only seldom.

4. *Endothelial cell. (Endothelial leucocyte, transitional cell.)*—This type of cell is supposed to be derived from the endothelial lining of the meninges. It is present in large numbers in the fluid of acute meningitis, especially in the fluid of meningococcus meningitis, and to a lesser degree in the fluid of subacute inflammatory processes of the meninges.

The endothelial cell is larger in size than a large lymphocyte. It contains a small, round, or oval, eccentrically placed nucleus, and a large amount of weakly staining protoplasm. The protoplasm often looks transparent and in places even seems to contain vacuoles.

Under the heading of endothelial leucocyte may be included the transitional cell, which is a large cell with indentated or horse-shoe shaped nucleus. Many authors consider the transitional cell as an endothelial leucocyte.

The Gitterzelln of the German authors is an endothelial cell in which the protoplasm is subdivided by fissures.

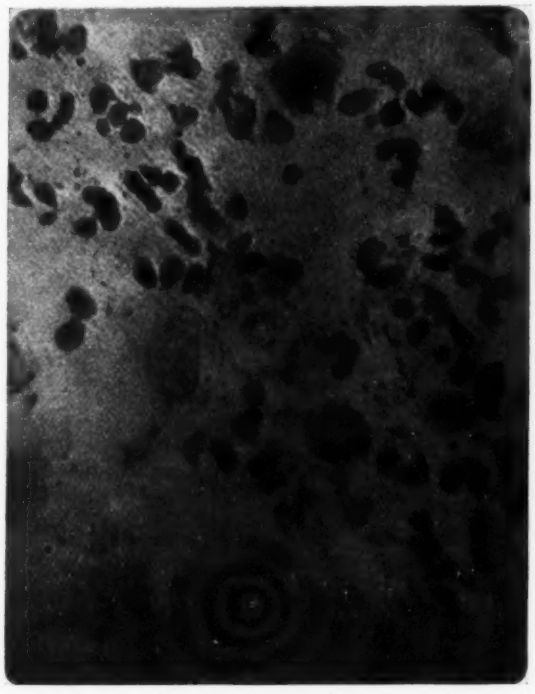

Fig. 2.—Direct smear of cerebrospinal fluid from another case of meningococcus meningitis.

5. *Phagocyte.*—In meningococcus meningitis and less frequently in other meningitides large cells containing smaller cells inside are seen in the fluid. The presence of phagocytes is not always an indication of the phagocytic action on the cells and has no prognostic significance.

6. *Fibroblast.*—This cell which is derived from connective tissue is present in chronic inflammations of the meninges. The cell body is elongated, pointed at both ends and contains a small round or oval nucleus.

7. *Plasma Cell.*—This cell which is found in old standing processes such as general paresis, is the size of a large lymphocyte or a little larger. It contains a granule and a deep-staining nucleus placed eccentrically.

8. *Erythrocyte.*—In fluid from meningeal hemorrhage, in all acute inflammatory processes and whenever blood has been obtained by an unsuccessful spinal puncture red blood cells are found in the fluid.

9. *Tumor and cyst cells.*—Sarcomatous cells may appear in the fluid of a case of sarcoma of the meninges. Echinococci have been found in the fluid from cases of echinococcus cysts. Sicard reported a case where actinomycotic granules were found in the fluid.

10. *Unclassified cells.*—In all destructive processes of the meninges, cells are met with in the cerebrospinal fluid in all stages of degeneration. The cells vary in size and shape and simply indicate destruction of the meninges.

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## EDITORIALS

### *Further Studies on Pellagra*

THE World War gave frequent opportunity for the study of pellagra, especially in Egypt and Asia Minor. In the fall of 1915 some 4,000 Armenians, men, women and children, who had been driven from their homes by the Turks and who had been wandering under great hardships in the mountainous regions of Syria, were collected by the British and carried to Port Said. They spent the winter of 1915-1916 in this camp on a restricted diet in which the gross protein per man per day amounted to 64 grams, while the available protein was only 51.5 grams and the biologic value of the protein only 23. By the spring of 1916 ten per cent of these refugees had developed pellagra. They were immediately supplied with an antipellagrous ration furnishing 99.3 grams of gross protein, 83 grams of available protein and 59.15 as the biologic value of the protein. On this diet they rapidly recovered.

Wilson[1] has gone quite minutely into the dietaries upon which people have developed pellagra, as well as upon antipellagrous rations, and has quite convinced himself that pellagra is not due to the absence from the food of a specific vitamin. He is equally convinced that this disease is not due to a deficiency in fat or to improper fats in the food. He points to the fact that the rise in the price of foodstuffs has restricted the supply of proteins much more than it has that of either carbohydrates or fats. He says: "That a deficiency in the amount and quality of the food is of primary importance in producing the disease, has been pointed out by the majority of observers from Casal onwards; also the reciprocal fact that a generous diet will cure the disease." All the pellagrous diets are deficient in proteins, and this is quite independent of whether or not they contain maize or maize products. In a few instances, while the ration has contained enough protein, the protein has been of inferior biologic value or it has failed of digestion and absorption.

Wilson concludes his rather exhaustive study of the dietary of this disease as follows: "(1) Pellagra is the ultimate result of a deficient supply of protein. (2) The sufficiency or insufficiency must be judged of by the biologic value of the protein, estimated on the amount available for assimilation and not on the gross protein; 40 may be taken as the minimum safe value for this factor. Below this, cases are likely to occur in the affected community; owing however to the great normal variations in the minimum protein requirement, many individuals, the biologic value of whose daily protein intake is as low as 20, will escape the disease, while it is possible that some, with a value above 40, may become affected. (3) The deficiency of protein may be: (a) *Primary,* in which the supply is insufficient for the individual requirement or, when, owing to the indigestible character of the food, a somewhat restricted supply cannot be utilized to the normal extent. (b) *Secondary,* in which owing to digestive disturbances, or other causes, the supply of protein cannot be assimilated. (4) In accordance with conclusion 3, three types of pellagra may be distinguished etiologically: (a) The common type seen in poverty or deficient food from other causes. (b) Relapse cases in which, owing to permanent defects—the result of a previous attack of pellagra—the protein supply is inefficiently utilized. (c) Cases in which a disease of the digestive organs due to other causes than insufficient feeding, leads to defective utilization of the protein intake. (5) That indicanuria is an important indication of the loss of protein in the intestine, the amount present being sometimes sufficient to account for the loss to the body of a large proportion of the protein intake. (6) Indicanuria is closely related to the deficiency of gastric hydrochloric acid. This is due to two causes: (a) The resulting invasion of the intestine with bacteria. (b) The absence of the normal stimulus to the secretion of pancreatic juice, whereby less protein is completely digested and more is lost in the large intestine. (7) Labor raises the level of protein requirement, an effect which is increased greatly by a deficient energy supply. Labor is therefore a factor in the causation of pellagra in a community whose protein supply is on the border-line between sufficiency and insufficiency. (8) There

is evidence which seems to suggest that a deficiency of cholesterol may be related to some of the symptoms."

Voegtlin[2] has prepared a map showing the present-day geographical distribution of pellagra, and Roberts[3] states that, although exact statistics are not available, there have been in this country from 1902 to 1920, 50,000 deaths from this disease. In 1916 pellagra ranked fourth as a cause of death in Mississippi, third in Alabama, and second in South Carolina. The most severe years since 1900 have been those between 1911 and 1916. There has been since 1917 a marked decrease both in prevalence and in severity of the disease. In some communities the decrease in number of cases has amounted to seventy-five per cent. Roberts thinks that, while there is a tendency to decrease in the number of cases, there is a tendency to increase in chronicity. According to this well-known authority on this disease, pellagra as seen in the South today is in most instances exceedingly mild compared with the same disease as seen twenty years ago. Acute pellagra is becoming rare, while mild cases are more in evidence. Bigland,[4] basing his observations upon Turkish prisoners seen during the War, concludes that pellagra is most frequently seen among the underfed, but is occasionally seen among the well-fed. In the latter instance, although the food eaten is in proper quality and quantity, there is something wrong with its digestion, absorption or assimilation. Enright[5] bases his statements upon studies of the disease as seen among German prisoners who were captured in the East and held in Egypt. Enright thinks that there is something more than a dietary deficiency in the causation of this disease. He found that the German prisoners apparently had an abundant ration and were not subjected to any undue hardship. He asks himself why, under these conditions, the German prisoners suffered from pellagra while the English soldiers, with no better food and doing much harder work, escaped. Enright's explanation when he attempts to answer this question is not very satisfactory. He is sure there was no food deficiency and he is inclined to the opinion that there must have been something wrong with the internal secretions, but what secretion was involved and what was wrong with it he is unable to tell. Year by year, and with the accumulation of observations, it seems that the weight of evidence showing that pellagra is due to an unbalanced diet, and especially to one poor in proteins of high biologic value, grows, and we are ready to agree with Voegtlin, who says: "(1) The hypothesis that there is a causal relation between pellagra and a restricted vegetable diet has been substantiated by direct proof to this effect and has led to results of considerable practical and scientific value. (2) The metabolism in pellagra shows certain definite changes from the normal, which point to decreased gastric secretion and increased intestinal putrefaction. (3) In the treatment and prevention of pellagra, diet is the essential factor. The disease can be prevented by an appropriate change in diet without changing the other sanitary conditions. (4) A diet of the composition used by pellagrins prior to their attack by the disease leads to malnutrition and certain pathologic changes in animals, resembling those found in pellagra. A typical pellagrous dermatitis

has not been observed in animals. Pellagra symptoms have been produced in man by the continued consumption of a restricted vegetable diet.''

Roberts emphasizes the importance of rest and diet in the treatment of this disease, and suggests the alterative prescription of food, fat, and flat.

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—*V. C. V.*